# Dimensional phenotypes and molecular genetic studies of obsessions, compulsions and tics

Hilga Katerberg

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Stellingen horend bij het proefschrift

# *Dimensional phenotypes and molecular genetics studies of obsessions, compulsions and tics*

van

*Hilga Katerberg*

1. Er zijn bij obsessieve-compulsieve stoornissen ten minste vier erfelijke symptoomdimensies te onderscheiden:
    1) Taboe
    2) Smetvrees/wassen
    3) Twijfel
    4) Symmetrie/verzamelen

2. Er is bij obsessieve-compulsieve stoornissen sprake van zowel erfelijke factoren voor obsessieve-compulsieve stoornissen in het algemeen als specifieke factoren voor een of meerdere symptoomdimensies. Daarom is het van belang om in toekomstig onderzoek onderscheid te maken tussen het syndroom als geheel en aparte symptoomdimensies.

3. Bij factoranalyse van de Yale-Brown Obsessive-Compulsive Scale symptom checklist heeft analyse op symptoomniveau slechts beperkte toegevoegde waarde ten opzichte van analyse op categorieniveau.

4. De symptomen uit de categorieën "gemengde obsessies" en "gemengde compulsies" zijn heterogeen en kunnen beter weggelaten worden bij genetische analyses.

5. Hoewel missense muaties in het epsilon sarcoglycaan gen in sommige families met myotone dystonie geassocieerd zijn met obsessieve-compulsieve stoornis, spelen zij op populatie niveau geen belangrijke rol in de etiologie van obsessief-compulsieve stoornis en het syndroom van Gilles de la Tourette.

6. Bij het moleculair genetisch onderzoek van obsessieve-compulsieve stoornis en het syndroom van Gilles de la Tourette heeft de interactie tussen genen en omgeving tot nog toe onvoldoende aandacht gekregen.

7. In de meeste publicaties waarbij grote groepen proefpersonen zijn betrokken ontbreekt de uitleg over hoe is omgegaan met missende waarden evenals informatie omtrent de aard van de missende waarden.

8. De gevoelsmatige afstand tussen Groningen en Amsterdam is afhankelijk van hoe vaak men deze afstand aflegt en waar men woont.

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*Voor mijn vader*

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Chapter 1

# Introduction

**Obsessive-Compulsive Disorder and Tourette syndrome**

Obsessive Compulsive Disorder (OCD) is a relatively common disorder with a lifetime prevalence of about 2-3% (Bebbington, 1998; Karno et al., 1988). The prevalence of this disorder is remarkable similar between different populations, with the exception of Taiwan which has a low prevalence for all psychiatric disorders (Weissman et al., 1994).

The first description of the symptoms nowadays categorized as obsessive compulsive disorder was provided in 1878 by Carl Westphal, who published his views on what he described as "zwangsvorstellungen" (Westphal, 1878). As key features of this condition he considered the presence of intruding ideas or images, the impossibility to suppress these ideas or images, and the realization that they are excessive and/or unreasonable. In addition, he stressed the absence of affective symptoms.

The key features of the current OCD diagnosis are the presence of recurrent and persistent thoughts, impulses or images (obsessions), which are often accompanied by the need to perform repetitive behaviors or mental acts (compulsions) to alleviate the anxiety caused by the obsessions (American Psychiatric Association, 1994). Diagnostic criteria for OCD as described in the Diagnostic and Statistical Manual of Mental Disorders version IV (DSM-IV) are summarized in table 1.

It has been suggested that there is a spectrum of disorders that show similarity to OCD based on symptomatology, comorbidity, aggregation of these disorders within families and similar neurocircuitry and treatment response (Hollander et al., 2005). One of these putative OCD spectrum disorders is Gilles de la Tourette syndrome (GTS).

In 1885, Gilles de la Tourette published a report in which he described 9 patients with involuntary movement, echolalia and copropraxia, which he considered the key symptoms of the syndrome that currently bears his name (Gilles de la Tourette, 1885). Nowadays, GTS is described as a neuropsychiatric disorder characterized by multiple motor tics and at least one vocal tic. Current DSM-IV-TR criteria for Tourette syndrome are summarized in table 2.

Table 1. DSM-IV diagnostic criteria for OCD.

A. Either obsessions or compulsions

Obsessions as defined by *(1), (2), (3), and (4)*:

1. Recurrent and persistent thoughts, impulses, or images that are experienced, at some time during the disturbance, as intrusive and inappropriate and that cause marked anxiety or distress.
2. The thoughts, impulses, or images are not simply excessive worries about real-life problems.
3. The person attempts to ignore or suppress such thoughts, impulses, or images, or to neutralize them with some other thought or action.
4. The person recognizes that the obsessional thoughts, impulses, or images are a product of his or her own mind (not imposed from without as in thought insertion).

Compulsions as defined by (1) and (2):

1. Repetitive behaviors (e.g., hand washing, ordering, checking) or mental acts (e.g., praying, counting, repeating words silently) that the person feels driven to perform in response to an obsession, or according to rules that must be applied rigidly.
2. The behaviors or mental acts are aimed at preventing or reducing distress or preventing some dreaded event or situation; however, these behaviors or mental acts either are not connected in a realistic way with what they are designed to neutralize or prevent or are clearly excessive.

B. At some point during the course of the disorder, the person has recognized that the obsessions or compulsions are excessive or unreasonable. Note: This does not apply to children.

C. The obsessions or compulsions cause marked distress, are time consuming (take more than 1 hour a day), or significantly interfere with the person's normal routine, occupational (or academic) functioning, or usual social activities or relationships.

D. If another Axis I disorder is present, the content of the obsessions or compulsions is not restricted to it (e.g., preoccupation with food in the presence of an Eating Disorder; hair pulling in the presence of Trichotillomania; concern with appearance in the presence of Body Dysmorphic Disorder; preoccupation with drugs in the presence of a Substance Use Disorder; preoccupation with having a serious illness in the presence of Hypochondriasis; preoccupation with sexual urges or fantasies in the presence of a Paraphilia; or guilty ruminations in the presence of Major Depressive Disorder).

E. The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

Depending on the diagnostic criteria used, prevalence rates of GTS ranging between 1:100 and 1:10.000 have been described (Keen-Kim and Freimer, 2006).

Complex tics in GTS may be difficult to distinguish from compulsions in Obsessive-Compulsive Disorder. Moreover, there is considerable comorbidity between OCD and GTS.

Table 2. DSM-IV diagnostic criteria for Gilles de la Tourette's syndrome.

A. Both mulitple motor tics and one or more vocal tics must be present at the same time, although not necessarily concurrently;

B. The tics must occur many times a day (usually in bouts) nearly every day or intermittently over more than 1 year, during which time there must not have been a tic-free period of more than 3 consecutive moths;

C. The age at onset must be less than 18 years;

D. The disturbance must not be due to the direct physiological effects of a substance (e.g. stimulants) or a general medical condition (e.g. Huntington's disease or postviral encephalitis).

*Neurobiological models of OCD and Gilles de la Tourette's syndrome*

It has been hypothesized that the etiology of OCD and GTS involves a disturbance of the cortico-striato-thalamo-cortical circuitry (Carlsson, 2000; Leckman et al., 2006; Singer, 2005).

The presence of an imbalance between the direct and indirect striatothalamic pathway has been hypothesized (illustrated in figure 1). This hypothesis states that there is an increased activity of the *direct striatothalamic pathway,* which is activated by dopamine DRD1 receptors of neurons in the matrix of the dorsolateral striatum. These neurons exert an inhibitory GABA-ergic influence on neurons in the Globus pallidus interna and pars reticulare of the substantia nigra, which exert an excitatory influence on thalamic neurons mediated by GABA. These neurons have excitatory projections to the cortex. The direct striatocorticothalamic pathway thus provides a positive behaviorally stimulating striatopallidothalamocortical feedback loop.

There is a decreased activity of the *indirect striatothalamic pathway* activated by dopamine DRD 2 receptors in the ventromedial striatum. These neurons exert an inhibitory GABA-ergic influence on neurons of the globus pallidus externa, which in their turn exert an inhibitory GABA-ergic influence on the subthalamic nucleus. Neurons in the subthalamic nucleus have excitatory glutamatergic projections to the thalamus. The indirect striatothalamic pathway thus provides a behaviorally inhibiting negative striatopallidothalamocortical feedback loop.

The hypothesized imbalance between inhibitory direct pathway and the excitatory indirect pathway in GTS and OCD results in a reduced inhibitory influence of Gpi neurons projecting to the thalamus (Leckman et al., 2006; Saxena et al., 1998).

There is evidence that there are five distinct parallel cortico striatal pathways: the motor circuit, the oculomotor circuit, the dorsolateral prefrontal circuit, the lateral orbitofronal circuit and anterior cingulate circuit (Singer and Minzer, 2003). It has been hypothesized that these pathways may be involved in the etiology of specific tics and/or obsessions and/or compulsions. For example, the motor circuit may be involved in tics, the oculomotor circuit may be involved in ocular tics and the dorsolateral prefrontal circuit and the anterior cingulate pathway may be involved in OCD (Singer and Minzer, 2003).

In addition, it has been hypothesized that there might be a more general increased glutamatergic activity in OCD involving cortical glutamatergic hyperactivity as well as an increased activity of both the direct stimulatory and the indirect inhibitory striatothalamic pathways (Carlsson, 2000). Increased cortical glutamatergic activity could be mediated by stimulation of the cortex by serotonin e.g. by the nucleus raphe.

Some children with childhood onset OCD and/or tic disorder develop exacerbations of symptoms after group-A beta-haemolytic streptococcal (GAS) infection. These cases of OCD and/or tics are identified by the acronym PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections). According to the auto-immune hypothesis, OCD and tics in this subgroup may occur as a result of post-streptococcal autoimmunity, in which auto-antibodies to GAS cross-react with neurons in the basal ganglia (Snider and Swedo, 2004).

From the hypotheses described here above, interactions between several neurotransmitter systems and, in a subset of patients, auto-immune mechanisms may be involved in the etiology of OCD.

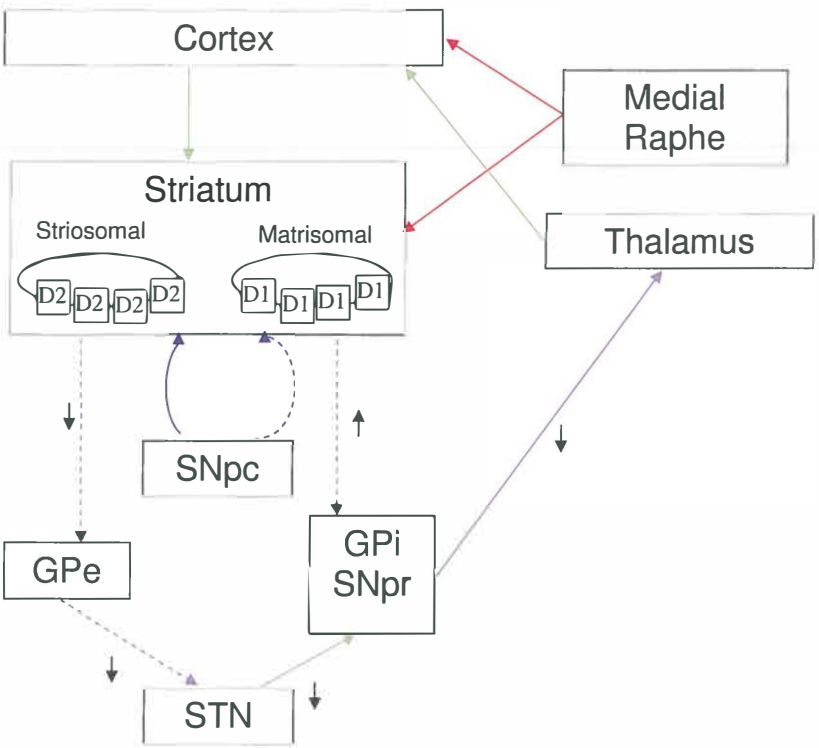


Figure 1 Neurobiological model of OCD and GTS.
GPe=Globus Pallidus externa; GPi=Globus Pallidus interna; SNpr=Substantia Nigra pars reticulare; SNpc=Substantia Nigra pars reticular; STN=Subthalamic Nucleus. Arrows with a solid line indicate a stimulating action; arrows with a broken line indicate an inhibitory action. Arrows in blue indicate dopaminergic neurotransmission; arrows indicated in green show glutamatergic neurotransmission, arrows indicated in pink indicate GABA-ergic transmission and arrows in red indicate serotonergic transmission.

## The role of genetics in the etiology of OCD and GTS

### The genetics of OCD

When Westphal described the disorder currently known as OCD, he already acknowledged a probable familial component in its etiology:

*"Auch die Zwangvorstellungen scheinen besonders häufig bei Personen auftreten, in deren Familie eine Disposition zu Neurosen und Psychosen herrscht"*

(Westphal, 1878)

However, it was not until last decades, that family studies were performed, which showed that OCD can be familial (Black et al., 1992; do Rosario-Campos et al., 2005; Grados et al., 2001; Hanna et al., 2005b; Nestadt et al., 2000b; Pauls et al., 1995). First degree family members of OCD patients have about a 4-5 times increased risk for OCD compared to family members of healthy controls (Nestadt et al., 2000b; Pauls et al., 1995). Some family studies suggest that comorbid tic disorder (Pauls et al., 1995) and early-onset (Nestadt et al., 2000b) are associated with a more familial form of the disorder. However, in family studies, no distinction can be made between the contributions of genetic and family environmental factors to the aggregation of OCD within families. Twin studies provide a valuable tool to distinguish the contribution of genetic factors (heritability) and common environmental factors to the familial aggregation of OCD by comparing symptom similarity within and between monozygotic (MZ) and dizygotic (DZ) twins. Twin studies in OCD have found heritability estimates ranging between 26% and 58% depending on the age- and sex distribution in the sample (Clifford et al., 1984; Hudziak et al., 2004; Jonnal et al., 2000, reviewed by van Grootheest et al., 2005). Thus, twin studies indicate that there is a considerable genetic component in the etiology of OCD. Heritability estimates of OC symptoms are in the range of 45% to 65% for studies in children and 27% to 47% for studies in adults (van Grootheest et al., 2005).

The mode of transmission of a disorder is studied by segregation analysis in pedigrees. Segregation studies in OCD suggest underlying genetic mechanisms that most likely involve genes of major effect complicated or modified by a multigenetic background and sex (Alsobrook et al., 1999; Cavallini et al., 1999; Nestadt et al., 2000a).

### The genetics of GTS

When Gilles de la Tourette provided the case descriptions of patient with GTS in 1885 he cited Trousseau who recognized the heritability of tics:

*"Ces tics sont bien souvent héréditaires"* (Trousseau, 1868).

Family studies show that 9.8-15% of relatives of GTS patients suffer from GTS and 15-20% from tics (Keen-Kim and Freimer, 2006; Pauls, 2003). Concordance rates for GTS

in monozygous twins range from 50-70% compared to about 9% in heterozygous twins. Concordance rates for chronic motor tics are 77% and 25% in monozygous and dizygous twins respectively. This suggests that GTS is highly heritable, but that environmental factors are also involved in the etiology of GTS. Early segregation analyses suggested an autosomal dominant inheritance model, whereas more recent segregation analyses support a complex additive inheritance model with a significant multifactorial background (Keen-Kim and Freimer, 2006; Pauls, 2003).

GTS and other Tic Disorders (TD) are more common in OCD patients than in the general population (Grados et al., 2001; Holzer et al., 1994; Leonard et al., 1992; Zohar et al., 1997) and in their first-degree relatives when compared to relatives of non-OCD controls (Hanna et al., 2005b; Pauls et al., 1995, 1991, 1986). Reversely, OCD is more common in first-degree relatives of patients with GTS and other TD, independently of whether the proband has co-morbid OCD or not (Grados et al., 2001). Family studies suggest that there are three types of OCD: 1) a familial type related to TD, 2) a familial type not related to TD, and 3) a non-familial subtype (Pauls et al., 1995). Furthermore, tics were significantly associated with OCD in a longitudinal study (Peterson et al., 2001a). These findings suggest that at least some forms of OCD and GTS share one and probably more genetic etiological factors. Both OCD and GTS are complex genetic disorders, caused by multiple genes, environmental factors and probably gene-gene and gene-environment interactions. Individual susceptibility loci for OCD and GTS are probably of modest effect and are neither sufficient nor necessary to develop the disorder. The genetics of OCD and GTS is characterized by genetic heterogeneity (i.e. different genes will contribute to the development of the disorder in different patients) and allelic heterogeneity (i.e. different alleles within the same gene contribute to the development of the disorder).

**Molecular genetic studies in OCD and GTS**

The two main approaches in the molecular genetic studies of OCD and GTS are linkage and association studies.

*Linkage studies* investigate the segregation of polymorphic DNA markers and the disease within families. If the co-segregation of the allele under investigation and the disease occurs more or less frequently than expected by chance, this may indicate that allele confers a susceptibility or protects for the disease or because it is linked to a susceptibility locus for the disease (Borecki and Province, 2008).

*Association studies* investigate whether specific alleles or haplotypes of polymorphic DNA marker(s) are more common in patients compared to controls, i.e. whether specific alleles are correlated with the disease. An advantage of association studies over linkage studies is that association studies are more powerful to detect susceptibility genes of small effect size (Borecki and Province, 2008).

In case-control association studies, allele frequencies of polymorphic markers in patients are compared with those in non-related unaffected controls. When cases and controls originate from different (sub) populations with different genetic backgrounds, differences in allele frequencies between cases and controls may be due to difference in genetic background rather than linkage between the marker and the disease susceptibility locus. This phenomenon is called population stratification and may bias the results of case-control association studies when patients and controls are recruited from different subpopulations (Thomas and Witte, 2002). To circumvent the problem of population stratification, family-based methods have been developed in which control alleles are obtained from family members to ensure that the control alleles are from the same (sub) population. Several methods to test for association in family-based association studies have been developed, including the Haplotype-based Haplotype relative risk (HHRR) test (Falk and Rubinstein, 1987); the Transmission Disequilibrium Test (TDT; Spielman et al., 1993) and the Sibship TDT (STDT; Spielman and Ewens, 1998). For an overview see Schulze and McMahon (2002).

Both linkage and association studies can use a candidate gene approach or a genome wide approach. Association studies are more powerful than linkage studies to identify genes of modest effect. Linkage studies however, have been successfully used to identify genes with large effect (Risch and Merikangas, 1996).

Candidate genes are genes suspected to have an influence on disease based on their putative role in the etiology of the disease (functional candidate genes) or their chromosomal position in a region identified as a susceptibility region in linkage studies (positional candidate genes).

**Genome-wide studies**

In OCD three genome wide linkage studies have been performed. Hanna et al. investigated fifty-six persons from 7 OCD families with early-onset OCD (<18 years) and found evidence suggestive for linkage on chromosome 9p24 (Hanna et al., 2002). Linkage in this region was subsequently replicated with a linkage study of 9p24 markers in an independent sample of 193 subjects from 50 OCD pedigrees (Willour et al., 2004). The highest LOD score in this study was 2.52 obtained with non-parametric analysis. The highest LOD score with a parametric analysis was 2.26, using a dominant inheritance model.

However, this finding was not replicated by another linkage study (Hanna et al., 2007). The second genome-wide linkage study found suggestive evidence for linkage at chromosome 10p15 indicating the *ADAR 3* gene (adenosine deaminase acting on RNA member 3) as a positional candidate gene for OCD (Hanna et al., 2007). The most recent study in 219 families found evidence suggestive for linkage was found on chromosome 3q27-28, 7p, 6q, 1q and 15q and stratification by gender and fine mapping provided evidence of a susceptibility region at chromosome 11p15 in males (Bienvenu et al., 2008; Shugart et al., 2006).

Several linkage studies have been performed in GTS (Barr et al., 1999a; Curtis et al., 2004; Merette et al., 2000; Pakstis et al., 1991; Simonic et al., 1998; TSAICG, 1999). Linkage studies in large pedigrees show evidence linkage to chromosomal regions 5p13-q11.2, 5q, 10, 11q23, 17q25, 3q and 9q (Barr et al., 1999a; Curtis et al., 2004; Merette et al., 2000; Verkerk et al., 2006). A study in affected sib pairs identified a susceptibility region at chromosome 4q and 8p (TSAICG, 1999). In addition, a whole genome association analysis yielded evidence for chromosome 2, 8q, 11q, 14q, 20q and 21q (Simonic et al., 1998). The most recent studies assessed both affected sib pairs and multigenerational families and found evidence suggestive for linkage at chromosome 2p, 3p, 3q, 4p ,6p, 10p 15p, 21p and Xp in the affected sib pairs, 2p and 5p in the multigenerational families (TSAICG, 2007). For region 11q23-24, evidence suggestive for linkage was obtained in two studies (Merette et al., 2000; Simonic et al., 1998).

*Evidence for shared susceptibility regions for OCD and GTS*

There is no overlap between the putative susceptibility regions for OCD and GTS identified by whole genome linkage scans. This may be due to the fact that, with the exception of the study of Hanna et al (2003), probands with GTS were excluded in the genome wide linkage studies of OCD. However, several reports of cytogenetic anomalies in patients with OCD/GTS/tics have been described. These anomalies and

the genes disrupted by these anomalies may provide clues to the loci and genes involved in OCD and GTS.

A de novo duplication of the long arm of chromosome 7 (dup(7)(q22.1-31.1)) has been described by Petek et al. in a patient with GTS, reduced speech development and depression (Petek et al., 2001). The *IMMP2* gene, a gene encoding a human homologue of the yeast mitochondrial membrane peptidase subunit 2 was disrupted by this duplication.

Verkerk et al (2003) described an insertion/translocation between chromosome 2 and 7 (der7)ins(7;2)(q35-q36;p21-p23) which was transmitted from a father with OCD to his daughter and son. Both suffered from OCD, GTS, mental retardation and growth retardation. This insertion/translocation disrupted the contactin-associated protein 2 gene (*CNTNAP2*) gene, which encodes a membrane located in nodes of Ranvier of axons. These findings suggest that the *CNTNAP2* and *IMM2PL* gene may be involved in GTS.

Other studies showed a translocation between chromosome 7 and 18 (t(7;18)(q22.1-q22.3) in a family with GTS and OCD (Boghosian-Sell et al., 1996), a chromosome 18 inversion in a patient with chronic tics and OCD (State et al., 2003), and a translocation between chromosome 2 and 18 (t(2;18)(p12,q22) in a patient with severe OCD (Cuker et al., 2004) findings suggest susceptibility regions for OCD and/or GTS on chromosome 7q and 18q22.

**Candidate gene studies in OCD and GTS**

Based on the afore-mentioned described neurobiological mechanisms thought to be involved in the etiology of OCD and GTS, genes in the serotonergic, dopaminergic, glutamatergic and GABA-ergic pathways as well as genes involved in auto-immune reactions can be considered as functional candidate genes for OCD and GTS.

Serotonergic system

The serotonergic system is involved in behavioral functions such as fear, perception, memory and mood (Berger et al., 2009; Serretti et al., 2006). A role for the serotonergic system in the etiology of OCD has been postulated based on the efficacy of serotonin reuptake inhibitors (SSRIs) in alleviating symptoms (Baumgarten and Grozdanovic, 1998) and on pharmacological challenge studies (Westenberg et al., 2007).

Since the serotonin transporter is the primary site of action of SSRI's, it is not surprising that the serotonin transporter gene (*5HTT* gene) is the most widely studied gene in OCD. Association studies investigating the *5HTT* gene in OCD and GTS are summarized in table 3. The *5HTT* gene has a polymorphic 44 bp insertion/deletion site in its upstream promoter region (HTTPR). The long allele of this polymorphism ("l" allele) is associated with about three times higher serotonin transporter expression levels than the short "s" allele (Heils et al., 1996). Two meta-analyses of association studies of the 5HTTPR S/L genotype and OCD were performed (Bloch et al., 2008b; Lin, 2007). One of these studies showed association of OCD with the SS genotype (Lin, 2007). The other study failed to show association with the 5HTTPR S/L polymorphism in the overall analysis, but the LL genotype was linked with OCD in family-based studies and in studies involving Caucasians and children (Bloch et al., 2008b). To our knowledge, only one study investigated the 5HTTP S/L polymorphism in GTS, with negative result (Cavallini et al., 2000).

The inconsistent results of the association studies of the 5HTTPR in OCD may have several causes. First, there is a functional polymorphism within the 5HTTPR. This A>G substitution, if present in the L allele, renders the activity of the L allele nearly equivalent to that of the S allele (Hu et al., 2006). Moreover, genotyping of the 5HTTP S/L polymorphism is prone to genotyping errors at PCR conditions with high magnesium concentration (Yonan et al., 2006). Therefore, the results of studies investigating the 5HTTPR insertion/deletion polymorphism should be considered with caution. Other functional variants in the *5HTT* gene including the rare I425L and a VNTR in intron 2 have been described. Future studies investigating the *5HTT* gene should involve genotyping of the newly identified polymorphism within the 5HTTPR as well as other functional variants within the 5HTT.

Table 3. Studies testing for association between the bi-allelic 44bp insertion/deletion polymorphisms in the serotonin transporter gene and OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Diagnostic criteria | Phenotype | Design | Test | Sub-group | Allelic p | Genotypic p | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Serotonin transporter gene (17q11.1-q12)** | | | | | | | | | | | |
| Billett et al., 1997 | Canadian | 72 | 72 | DSM-IV | OCD | CC | $\chi 2$ | | 0.906 | 0.155 | negative |
| Mc Dougle et al., 1998 | USA | 34( | | DSM-IV | OCD±GTS/tics | FB | TDT | | **< 0.03** | | L allele associated with OCD |
| Bengel et al., 1999 | German | 75 | 397 | DSM-IIIR | OCD, tics unknown | CC | $\chi 2$ | | **0.036** | 0.14 | LL genotype associated with OCD |
| Cavallini et al., 2000 | Italian | 52 | 63 | DSM-IV | GTS±OCD | CC | $\chi 2$ | | 0.929 | 0.77 | negative |
| Kinnear et al., 2000 | South African | 54 | 82 | DSM-IV | OCD, tics unknown | CC | $\chi 2$ | | 0.09 | 0.19 | negative |
| Frisch et al., 2000 | Israeli | 73 | 172 | DSM-IV | OCD without TD | CC | $\chi 2$ | | ns | ns | negative |
| Camarena et al., 2001 | Mexican | 115 | 136 | DSM-IV | OCD, tics unknown | CC | $\chi 2$ | | 0.211 | 0.32 | negative |
| | | 43 | | | | FB | TDT | | > 0.05 | | negative |
| | | 43 | | | | FB | HHRR | | 0.24 | | negative |
| Cavallini et al., 2002 | Italian | 180 | 112 | DSM-IV | OCD±tics | CC | $\chi 2$ | | 0.592 | 0.73 | negative |
| Di Bella et al., 2002b | Italian | 181 | 191 | DSM-IV | OCD±TD | CC | $\chi 2$ | | 0.133 | 0.228 | negative |
| Chabane et al., 2004 | French/ German | 116 | | DSM-IV | OCD, tics unknown | FB | TDT | | 0.36 | | negative |
| | French | 106 | 171 | | | CC | $\chi 2$ | | ns | | negative |
| Meira-Lima et al., 2004 | Brazilian | 77 | 202 | DSM-IV | OCD, tics unknown | CC | $\chi 2$ | | 0.08 | 0.1 | negative |
| Walitza et al., 2004 | German | 64 | | DSM-IV | OCD without GTS | FB | TDT | | 0.16 | | negative |
| Kim et al., 2005 | Korean | 124 | 171 | DSM-IV | OCD±tics | CC | $\chi 2$ | | 0.90 | 0.96 | negative |
| Denys et al., 2006b | Dutch | 156 | 134 | DSM-IV | OCD, tics unknown | CC | $\chi 2$ | | 0.607 | 0.787 | S allele and OCD associated |
| | | 100 | | | | | | Females | **0.014** | | in females |
| Perez et al., 2006 | Caucasian | 26 | 89 | DSM-IV | OCD, tics unknown | CC | $\chi 2$ | | | < 0.01 | SS genotype associated with OCD |
| Dickel et al., 2007 | USA | 59 | | DSM-IIIR | | FB | $\chi 2$ | | 0.24 | | L allele associated with |
| | | 32 | | | | | | Males | 0.73 | | OCD in females |
| | | 27 | | | | | | Females | **0.03** | | |
| Saiz et al., 2008 | Spanish Caucasian | 99 | 456 | DSM-IV | OCD, GTS unknown | CC | $\chi 2$ | | 0.894 | | negative |

CC=Case-control, FB=Family-based, TDT=Transmission Disequilibrium Test, HHRR= Haplotype-based Haplotype Relative Risk, ns=not significant.

Table 3 (continued). Studies testing for association between polymorphisms in the serotonin transporter gene and OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Diagnostic criteria | Phenotype | Polymorphism | Design | Test | Allelic p | Genotypic p | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Serotonin transporter gene (17q11.1-q12)** | | | | | | | | | | | |
| Hu et al., 2006 | USA/ Canadian | 169 | 253 | DSM-IIIR | OCD | 44bp ins/del promoter tri-allelic | CC | $\chi 2$ | **0.035** | **0.001** | $L_A$ allele and $L_AL_A$ genotype associated with OCD |
| | | 175 | | DSM-IIIR | OCD | 44bp ins/del promoter tri-allelic | FB | TDT | **<0.023** | | $L_A$ allele associated with OCD |
| Ohara et al., 1998a | Japanese | 15 | 106 | DSM-IV | OCD, tics unknown | VNTR intron 2 | CC | $\chi 2$ | 0.0326 | - | Stin2.12 allele associated with OCD |
| Baca-Garcia et al., 2007 | Spanish | 97 | 406 | DSM-IV | OCD, tics unknown | VNTR intron 2 | CC | | | **0.026** | Stin2.12 allele associated with OCD |
| Grados et al., 2007 | German | 149 | 397 | NR | OCD | 44bp ins/del promoter | CC | $\chi 2$ | 0.101 | 0.063 | negative |
| Wendland et al., 2007 | USA | 295 | 657 | DSM-IV | OCD±tics | 44bp ins/del promoter | CC | $\chi 2$ | ns | | negative |
| | | | | | | 44bp ins/del promoter tri-allelic (+$L_G$/$L_A$) | CC | $\chi 2$ | ns | | negative |
| | | | | | | VNTR intron 2 | CC | $\chi 2$ | ns | | negative |
| | | | | | | I425V/I425L | CC | $\chi 2$ | | | variant not present |
| | | | | | | P339L | CC | $\chi 2$ | | | variant not present |
| Wendland et al., 2008 | USA | 295 | 657 | NR | OCD | rs25532 | CC | $\chi 2$ | ns | | C allele rs16965628 associated with OCD |
| | | | | | | rs2020933 | CC | $\chi 2$ | ns | | |
| | | | | | | rs16965628 | CC | $\chi 2$ | **< 0.04** | | |
| | | | | | | VNTR intron 2 | CC | $\chi 2$ | ns | | |
| Saiz et al., 2008 | Spanish Caucasian | 99 | 456 | DSM-IV | OCD, GTS unknown | VNTR intron 2 | CC | $\chi 2$ | **0.007** | **0.033** | Stin2.12 allele associated with OCD in males |
| Baca-Garcia et al., 2005 | Spanish | 24 | 112 | NR | OCD, tics unknown | 44bp ins/del promoter | CC | $\chi 2$ | | | LL genotype associated with OCD |

NR=not recorded, CC=Case-control, FB=Family-based, TDT=Transmission Disequilibrium Test, ns=not significant.

*Serotonin receptors*

Association studies of the serotonin receptor genes in OCD and GTS are summarized in table 4-6. The serotonin 1Dβ receptor gene (*HT1Dβ* gene) is a candidate gene in OCD because the therapeutic effect of SSRI's in OCD may involve the desensitization of serotonin 1Dβ terminal autoreceptors on serotonergic neurons (Blier and de Montigny, 1998). The initially reported association between the silent G861C polymorphism in the *5HT1Dβ* gene and OCD (Mundo et al., 2000, 2002) and males with OCD (Kim et al., 2009) could not be replicated in several other studies (Camarena et al., 2004; Denys et al., 2006b; Di Bella et al., 2002a; Dickel et al., 2007; Hemmings et al., 2003; Walitza et al., 2004). Furthermore, no evidence was found for an association between the functional T371G polymorphism in the *5HT1Dβ* gene and OCD, possibly because of the low number of informative families (n=5) (Mundo et al., 2000). Genes encoding 5HT2-like receptors such as the serotonin 2A receptor (*5HT2A* gene ) and the serotonin 2C receptor *5HT2C* gene might be involved in OCD since serotonin exerts its function in the orbitofrontal cortex, a region shown to be implicated in the etiology of OCD, mainly by 5HT-2 like receptors (Blier and de Montigny, 1998). Three studies found an association between a promoter polymorphism in the *5HT2A* gene and OCD (Enoch et al., 2001, 1998; Walitza et al., 2002), two of which in females only (Enoch et al., 2001; Walitza et al., 2002). This association was not replicated in three other studies (Denys et al., 2006b; Saiz et al., 2008; Tot et al., 2003). The association found between the C516T polymorphism of the *5HT2A* gene (Meira-Lima et al., 2004) and OCD has not yet been replicated by other studies. Association studies of the T102C polymorphism in the *5HT2A* gene, the Cys23Ser polymorphism in the *5HTR2C* gene and the C178T polymorphism in the *5HT3A* gene yielded negative results (Cavallini et al., 1998; Dickel et al., 2007; Frisch et al., 2000; Meira-Lima et al., 2004; Mössner et al., 2007a; Nicolini et al., 1996; Saiz et al., 2008; Walitza et al., 2004). In GTS, the *5HT3A* and *5HT3B* receptors are the only serotonin receptors studied in association analysis, with negative result (Niesler et al., 2005).

Table 4. Association studies investigating the serotonin receptors in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Diagnostic criteria | Diagnosis | Polymorphism | Design | Test | Allelic p | Genotypic p | conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **5HT1DB gene (6q13)** | | | | | | | | | | | |
| Mundo et al., 2000 | Canadian | 67 | | DSM-IV | OCD, tics unknown | G861C | FB | TDT/STDT | **<0.006** | | G allele associated with OCD |
| Mundo et al., 2002* | Canadian | 121 | | DSM-IV | OCD±GTS/tics | G861C | FB | TDT | **0.023** | | G allele associated with OCD |
| Di Bella et al., 2002a | Italian | 79 | | DSM-IV | OCD±tics | G861C | FB | TDT | 0.13 | | negative |
| Hemmings et al., 2003 | South African | 71 | 129 | DSM-IV | OCD, tics unknown | G861C | CC | $\chi 2$ | 0.621 | 0.840 | negative |
| Camarena et al., 2004 | Mexican | 72 | | DSM-IV | | G861C | FB | FBAT | 0.901 | | negative |
| Walitza et al., 2004 | German | 64 | | DSM-IV | OCD without GTS | G861C | FB | TDT | 0.19 | | negative |
| Denys et al., 2006b | Dutch | 141 | 117 | DSM-VI | OCD, tics unknown | G861C | CC | $\chi 2$ | 0.647 | 0.866 | negative |
| Dickel et al., 2007 | USA | 54 | | DSM-IIIR | OCD±tics | G861C | FB | $\chi 2$ | 0.87 | | negative |
| Kim et al., 2009 | Korean | 167 | 107 | DSM-IV | OCD | G861C | CC | $\chi 2$ | **0.02** | **0.02** | G allele associated with OCD in males |
| Mundo et al., 2002 | Canadian | 121 | | DSM-IV | OCD±GTS/tics | T371G | FB | TDT | 0.705 | | negative |
| **5HT2C gene (Xq24)** | | | | | | | | | | | |
| Frisch et al., 2000 | Israeli | 73 | 172 | DSM-IV | OCD without TD | Cys23Ser | CC | $\chi 2$ | ns | ns | negative |
| Cavallini et al., 1998 | Italian | 109 | 107 | DSM-IIIR | OCD±tics | Cys23Ser | CC | $\chi 2$ | 0.617 | 0.612 | negative |
| **5HT3A gene (11q23.1-q23.2)** | | | | | | | | | | | |
| Mössner et al., 2007a | German | 75 ( | | DSM-IV | OCD without GTS | C178T | FB | TDT | 0.89 | | negative |
| Niesler et al., 2005 | German | 48 | 156 | DSM-IIIR | GTS, OCD unknown | -42C>T Exon 1 | MS/CC | $\chi 2$ | | 0.88442 | negative |
| | | 47 | 48 | | | IVS3 +7A>C | MS/CC | $\chi 2$ | | 0.34902 | negative |
| | | 48 | 47 | | | 576G>A | MS/CC | $\chi 2$ | | 0.54501 | negative |
| | | 48 | 47 | | | 831G>A/ Lys277Lys | MS/CC | $\chi 2$ | | 0.98284 | negative |
| | | 48 | 155 | | | 1377A>G | MS/CC | $\chi 2$ | | 0.05874 | negative |

*This study included the subjects of the study of Mundo et al., 2000
CC=Case-control, FB=Family based, MS=Mutation screening, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, STDT = sib Transmission Disequilibrium test, ns=not significant

Table 5. Association studies investigating the serotonin 2A receptor gene in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Diagnostic criteria | Diagnosis | Polymorphism | Design | Test | Subgroup | Allelic p | Genotypic p | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **5HT2A gene (13q14-21)** | | | | | | | | | | | | |
| Enoch et al., 1998 | USA/ Italian | 62 | 144 | DSM-IIIR | OCD, tics unknown | -1438G/A promoter | CC | $\chi 2$ | | **<0.05** | ns | A allele associated with OCD |
| Enoch et al., 2001 | USA | 101 | 138 | DSM-IIIR | OCD, tics unknown | -1438G/A promoter | CC | $\chi 2$ | Females | **0.015** | **0.02** | A allele associated with OCD |
| | | | | | | | | | Males | ns | ns | in women |
| Walitza et al 2002 | German | 55 | 123 | DSM-IV | OCD without GTS | -1438G/A promoter | CC | $\chi 2$ | | **0.048** | **0.046** | A allele associated |
| | | 29 | 108 | | | | | | Females | **0.03** | | with OCD |
| | | 26 | 115 | | | | | | Males | 0.31 | | in women |
| Tot et al., 2003 | Turkish | 58 | 83 | DSM-IV | OCD±tics | -1438G/A promoter | CC | $\chi 2$ | | | 0.85 | negative |
| | | | | | | T102C | CC | $\chi 2$ | | | 0.3 | negative |
| Dickel et al., 2007 | USA | 54 | | DSM-IIIR | OCD±tics | T102C | FB | TDT | | 0.14 | - | A allele |
| | | | | | | | | | OCD+tics | **0.05** | **-** | associated with OCD +tics |
| Nicolini et al., 1996 | Mexican | 67 | 54 | DSM-IIIR | OCD±tics | T102C | CC | $\chi 2$ | | 0.241 | 0.338 | negative |
| Frisch et al., 2000 | Israeli | 73 | 172 | DSM-IV | OCD without TD | T102C | CC | $\chi 2$ | | ns | ns | negative |
| Tot et al., 2003 | Turkish | 58 | 83 | DSM-IV | OCD±tics | T102C | CC | $\chi 2$ | | | 0.660 | negative |
| Hemmings et al., 2003 | South African | 71 | 129 | DSM-IV | OCD, tics unknown | T102C | CC | $\chi 2$ | | 0.24 | 0.100 | negative |
| Meira-Lima et al., 2004 | Brazilian | 78 | | DSM-IV | OCD, tics unknown | T102C | CC | $\chi 2$ | | 0.5 | 0.1 | negative |
| | | 79 | | DSM-IV | OCD, tics unknown | C516T | CC | $\chi 2$ | | **0.00007** | **0.0002** | C allele associated with OCD |
| Denys et al., 2006b | Dutch | 154 | 116 | DSM-IV | OCD, tics unknown | -1438G/A promoter | CC | $\chi 2$ | | 0.776 | 0.143 | negative |
| Saiz et al., 2008 | Spanish Caucasian | 99 | 420 | DSM-IV | OCD, tics unknown | -1438G/A promoter | CC | $\chi 2$ | | | ns | negative |
| | | | | | | T102C | CC | $\chi 2$ | | | ns | negative |

CC=Case-control, FB=Family based, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, ns=not significant

Table 6. Association studies investigating the serotonin 3B receptor gene and the tryptophan hydroxylase genes in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Phenotype | Diagnostic criteria | Polymorphism | Design | Test | Allelic p | Genotypic p | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HTR3B gene (11q23.1)** | | | | | | | | | | | |
| Niesler et al., 2005 | German | 48 | 162 | GTS, OCD | DSM-IIIR | -102delAAG Exon 1 | MS/CC | $\chi 2$ | | 0.26306 | negative |
| | | 49 | 62 | unknown | | IVS4 +11 C>T | MS/CC | $\chi 2$ | | 0.91738 | negative |
| | | 49 | 62 | | | IVS4 +12 A>G | MS/CC | $\chi 2$ | | 0.24198 | negative |
| | | 48 | 62 | | | 386A>C/Tyr129Ser | MS/CC | $\chi 2$ | | 0.10634 | negative |
| | | 49 | 63 | | | IVS6 + 72 A>G | MS/CC | $\chi 2$ | | 0.54936 | negative |
| | | 52 | 63 | | | 547G>A/Val183Ile | MS/CC | $\chi 2$ | | 0.20558 | negative |
| **TPH1 (11p15.1-p14)** | | | | | | | | | | | |
| Han et al., 1999 | USA Indians/USA, Finnish and Italian Caucasians | 88 | 142 | OCD | DSM-IIIR | T1095C | MS/CC | $\chi 2$ | ns | | negative |
| Frisch et al., 2000 | Israeli | 75 | 172 | OCD without TD | DSM-IV? | rs1800532 | CC | $\chi 2$ | ns | ns | negative |
| Walitza et al., 2004 | German | 64 | | OCD without GTS | DSM-IV | rs1800532 | FB | TDT | 0.52 | | negative |
| **TPH2 gene (4q31-32)** | | | | | | | | | | | |
| Delorme et al., 2006b | French/German/ Swedish/Swiss | 201 | 523 | OCD, tics unknown | DSM-IV | R441H | CC | | | | variant not present in cases and controls |
| Mössner et al., 2006 | German | 71 | | OCD without GTS | DSM-IV | rs4570625 | FB | TDT | 0.484 | | GC haplotype overtransmitted |
| | | | | | | rs4565946 | FB | TDT | 0.059 | | |
| Mössner et al., 2007b | German | 98 | 178 | GTS | NR | rs4570625 | CC | $\chi 2$ | 0.322 | | C allele and CC genotype associated with OCD |
| | | | | | | rs4565946 | CC | $\chi 2$ | **0.002** | | |

CC=Case-control, FB=Family based, MS=Mutation screening, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, ns=not significant

*Tryptophan hydroxylase (TPH)*

Tryptophan hydroxylase is the rate-limiting enzyme in the serotonin synthesis. Recently two isoforms of this enzyme have been identified, TPH1 and TPH2. TPH1 is mainly expressed in peripheral tissues whereas TPH2 is expressed in the Central Nervous System (Walther and Bader, 2003). Association studies investigating the A218C polymorphism in the *TPH1* gene reported negative findings (Frisch et al., 2000; Han et al., 1999; Walitza et al., 2004). A SNP at the 5' region of the *TPH2* gene was associated with GTS in a case-control study and showed a trend for an association with OCD in a family-based study (Mössner et al., 2006, 2007b). Moreover, associations of haplotypes composed of this 5' SNP and a SNP in intron 2 were observed for both OCD and GTS (Mössner et al., 2006, 2007b). The functional R441H variant of the *TPH2* gene was not found in OCD patients (Delorme et al., 2006).(see table 4).

Dopamine receptors and dopamine transporter gene (see table 7-10)

The dopaminergic system is involved in movement, reward and cognition (Schultz, 2007). It has been hypothesized that the dopaminergic system may play a role in the pathophysiology of OCD and that OCD may represent a hyperdopaminergic state, based on neuroimaging studies and the observation that dopamine-releasing substances such as bromocryptin and cocaine may induce obsessive-compulsive symptoms (Denys et al., 2004c). Furthermore, atypical antipsychotics may be beneficial in patients with OCD resistant to SSRI (Denys et al., 2004c; Fontenelle et al., 2007; Westenberg et al., 2007).

Association studies investigating the TaqI polymorphism of the dopamine receptor D2 gene (*DRD2)* gene in OCD found an association between the A2 allele of the TaqA1 polymorphism in the dopamine receptor 2 (*DRD2*) gene and OCD with comorbid tics and GTS (Nicolini et al., 1998). Other studies found an association with OCD in males only (Denys et al., 2006a) or had negative results (Billett et al., 1998). Both the TaqA1 and the H313H polymorphism in the DRD2 were found to be associated with GTS (Lee et al., 2005).

Studies of the Ser9Gly polymorphism in the DRD3 gene in both OCD and GTS did not find any evidence for association (Billett et al., 1998; Catalano et al., 1994; Diaz-Anzaldua et al., 2004; Nicolini et al., 1996). Similarly, studies of the dopamine transporter gene (*DAT* gene) in GTS and OCD, most of which studied the 40bp VNTR in exon 3 did not find evidence for association (Billett et al., 1998; Diaz-Anzaldua et al., 2004; Frisch et al., 2000; Hemmings et al., 2003; Miguita et al., 2007; Walitza et al., 2008; Yoon et al., 2007).

Longer alleles of the 48 bp repeat in the dopamine receptor 4 gene (*DRD4*) gene, especially the 7 repeat allele, has been found to be associated with both GTS and OCD (Camarena et al., 2007; Diaz-Anzaldua et al., 2004; Grice et al., 1996), albeit not in al studies (Billett et al., 1998; Frisch et al., 2000; Hemmings et al., 2003).

Association studies of the gene encoding Dopamine Beta hydroxylase (*DBH)* gene, which is involved in the conversion of dopamine into norepinephrin, in GTS patients had negative results. This gene has not been studied in OCD yet (Ozbay et al., 2006; Yoon et al., 2007).

Although the results are inconsistent, some genes in the dopaminergic system, e.g. the *DRD2* gene and *DRD4* gene may be involved in both OCD and GTS.

Table 7. Association studies investigating the dopamine receptor 2 gene in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Phenotype | Diagnostic Criteria | Polymorphism studied | Design | Test | Subgroup | Allelic p | Genotypic p | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DRD2 (11q22-q23)** | | | | | | | | | | | | |
| Nicolini et al., 1996 | Mexican | 67 | 54 | OCD±tics | DSM-IIIR | TaqIA | CC | $\chi 2$ | | 0.221 | 0.115 | A2 allele and A2A2 |
| | | 12 | | | | polymorphism | | | OCD+Tics | **0.033** | **0.017** | genotype associated with OCD+tics |
| Nicolini et al., 1998 | Mexican | 66 | 54 | | DSM-IV | TaqIA polymorphism | CC | $\chi 2$ | OCD+Tics | **0.014** | **0.001** | A2 variant associated with OCD+tics |
| Billett et al., 1998 | Canadian | 110 | 110 | OCD±tics | DSM-IV | TaqIA polymorphism | CC | $\chi 2$ | | 0.505 | 0.712 | negative |
| | | 110 | 110 | | DSM-IV | Ser311Cys (rs1801028) | CC | $\chi 2$ | | | 0.719 | negative |
| Denys et al., 2006a | Dutch | 139 | 135 | OCD, tics | DSM-IV | TaqIA | CC | $\chi 2$ | | 0.926 | 0.595 | A2 allele and A2A2 |
| | | 51 | 67 | unknown | | polymorphism | | | Males | **0.020** | **0.049** | genotype associated with |
| | | 88 | 66 | | | | | | Females | 0.672 | 0.692 | OCD in males |
| Diaz-Anzaldua et al., 2004 | French-Canadian | 110 | | GTS±OCD | DSM-IV | TaqIA polymorphism | FB | TDT | | ns | | Negative (trend for A1 allele) |
| Lee et al., 2005 | Taiwanese | 151 | 183 | GTS, OCD unknown | DSM-IV | TaqIA polymorphism | CC | $\chi 2$ | | **0.0065** | **0.004** | A1A1 genotype and A1 allele associated with GTS |
| | | 151 | 183 | GTS, OCD unknown | | H313H | | | | **0.0057** | **<0.0001** | CC genotype and allele frequency associated with GTS |
| Nöthen et al., 1994 | German | 61 | | GTS±OCD | DSM-IIIR | TagIA polymorphism | FB | HRR | | ns | | negative |
| Comings et al., 1997 | Non-Hispanic Caucasian | 227 | 63 | GTS, OCD unknown | DSM-IV | TagIA polymorphism | CC | $\chi 2$ | | | | A1A2 genotype and A1 allele associated with GTS |
| Comings et al., 1991 | Non-Hispanic whites | 147 | 314 | GTS, OCD unknown | DSM-IIIR | TagIA polymorphism | CC | $\chi 2$ | | **0.0001** | | A1 allele associated with GTS |

CC=Case-control, FB=Family based, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, HRR=Haplotype Relative Risk, ns=not significant

Table 8. Association studies investigating the genes encoding dopamine receptors type 1, 3 and 4 in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Phenotype | Diagnostic Criteria | Polymorphism studied | Design | Test | Subgroup | Allelic p | Genotypic p | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DRD1 (5q35.1)** | | | | | | | | | | | | |
| Chou et al., 2004 | Taiwanese | 148 | 83 | GTS,OCD unknown | DSM-IV | Snp115417 | CC | $\chi 2$ | | | | Variant not present |
| | | | | | | 5´utr a>g | CC | $\chi 2$ | | 0.627 | **0.016** | negative |
| Comings et al., 1997 | Non-Hispanic Caucasian | 227 | 63 | GTS,OCD unknown | DSM-IV | 5´utr a>g | CC | $\chi 2$ | | 0.51 | | More homozygostivy in GTS patients |
| **DRD3 (3q13.3)** | | | | | | | | | | | | |
| Nicolini et al., 1996 | Mexican | 63 | 54 | OCD±tics | DSM-III-R | Ser9Gly Msc 1 RFLP | CC | $\chi 2$ | | 0.926 | 0.595 | negative |
| Billett et al., 1998 | Canadian | 103 | 103 | OCD±tics | DSM-IV | Ser9Gly Msc 1 RFLP | CC | $\chi 2$ | | 0.092 | 0.19 | negative |
| Catalano et al., 1994 | Italian | 97 | 97 | OCD,tics unknown | DSM-III-R | Ser9Gly Msc 1 RFLP | CC | $\chi 2$ | | 0.095 | 0.19 | negative |
| Diaz-Anzaldua et al., 2004 | French-Canadian | 110 | | GTS±OCD | DSM-IV | *Msc*I polymorphism | FB | TDT | | ns | | negative |
| **DRD4 (11p15.5)** | | | | | | | | | | | | |
| Di Bella et al., 1996 | Italian | 157 | 162 | OCD,tics unknown | DSM-IIIR | 0 mutation (13bp del) exon 1 | CC | $\chi 2$ | | ns | ns | negative |
| Grice et al., 1996 | USA/ Canadian | 64 | | GTS/CMT | DSM-IV | 48bp VNTR exon 3 (7R allele) | FB | ETDT | | **0.002** | | 7R allele overtransmitted in GTS |
| Nicolini et al., 1998 | Mexican | 66 | 54 | ? | DSM-IV | 48bp VNTR exon 3 | CC | $\chi 2$ | OCD Tics | **0.014** | **0.001** | |
| Billett et al., 1998 | Canadian | 118 | 118 | OCD, tics unknown | DSM-IV | 48bp VNTR exon 3 | CC | $\chi 2$ | | **0.021** | 0.137 | negative |
| Hemmings et al., 2003 | South African | 71 | 129 | OCD | DSM-IV | 48bp VNTR exon 3 | CC | $\chi 2$ | | 0.07 | 0.175 | negative |
| Millet et al., 2003 | French Caucasian | 49 | 63 | OCD±tics | NR | 48bp VNTR exon 3 | CC | $\chi 2$ | | **10 -4** | **< 10 –4** | 2 allele protective |
| | | 55 | | | | 48bp VNTR exon 3 | FB | ETDT | | **0.003** | **0.003** | |

CC=Case-control, FB=Family based, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, ETDT=Extended Transmission Disequilibrium test, ns=not significant

Table 9. Association studies investigating the dopamine receptor 4 gene in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Phenotype | Diagnostic criteria | Polymorphism | Design | Test | Subgroup | Allelic p | Genotypic p | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DRD4 (11p15.5)** | | | | | | | | | | | | |
| Frisch et al., 2000 | Israeli | 71(75) | 172 | OCD | DSM-IV | 48bp VNTR exon 3 | CC | χ2 | | ns | ns | negative |
| | | | | without TD | | 48bp VNTR exon 3 | CC | χ2 | Non-Ashkenazi Jews | **0.04** | | undertransmission of 7R allele in Ashkenazi Jews |
| | | | | | | 7R allele | | | | | | |
| Diaz-Anzaldua et al., 2004 | French | 110 | | GTS±OCD | DSM-IV | 48bp VNTR exon 3 (7R allele) | FB | ETDT | | **0.033** | **0.038** | 7R allele overtransmitted |
| | Canadian | | | | | 120bpVNTR promoter | FB | ETDT | | ns | ns | |
| Camarena et al., 2007 | Mexican | 210 | 202 | OCD±tics | DSM-IV | 48bp VNTR exon 3 (4R allele) | CC | χ2 | | **0.0003** | | long alleles (5R-8R) associated with OCD |
| | | | | | | | | | Males | **0.0051** | | |
| | | | | | | | | | Females | **0.00275** | | |
| | | 86 | | | | | FB | ETDT | | 0.28 | 0.31 | |
| Yoon et al., 2007 | Non-Hispanic white | 226 | 236 | GST±ADHD | TSCSG/ DSM-IV | 48bp VNTR exon 3 | CC | χ2 | | ns | ns | negative, trend long alleles |
| | | | | | | 120bp upstream repeat | CC | χ2 | | ns | ns | negative |
| Tarnok et al., 2007 | Hungarian | 103 | 284 | GTS±OCD | DSM-IV | 48bp VNTR | CC | χ2 | | 0.624 | 0.419 | negative |
| | | | | | | | FB | ETDT | | 0.173 | | negative |
| | | | | | | 120bp duplication | CC | χ2 | | 0.473 | 0.740 | negative |
| | | | | | | | FB | ETDT | | 0.516 | | negative |
| | | | | | | -616C/G | CC | χ2 | | 0.744 | 0.255 | negative |
| | | | | | | | FB | ETDT | | 0.724 | | negative |
| | | | | | | -615A/G | CC | χ2 | | 0.800 | 0.827 | negative |
| | | | | | | -521C/T | CC | χ2 | | 0.875 | 0.707 | negative |
| | | | | | | | FB | ETDT | | 0.257 | | negative |
| Walitza et al., 2008 | German | 69 | | OCD without GTS | DSM-IV | 48bp VNTR exon 3 | FB | ETDT | | **0.03** | | trend for undertransmission of 4R allele |

CC=Case-control FB=Family based, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, ETDT=Extended Transmission Disequilibrium Test, ns=not significant.

Table 10. Association studies investigating the dopamine transporter gene, dopamine hydroxylase gene and the acid phosphatase locus 1 gene in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Phenotype | Diagnostic criteria | Polymorphism | Design | Test | Subgroup | Allelic p | Genotypic p | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DAT (5p15.3)** | | | | | | | | | | | | |
| Billett et al., 1998 | Canadian | 103 | 103 | OCD±tics | DSM-IV | 40bp VNTR | CC | $\chi 2$ | | 0.075 | 0.157 | negative |
| Hemmings et al., 2003 | South African | 71 | 127 | OCD,tics unknown | DSM-IV | 40bp VNTR | CC | $\chi 2$ | | 0.138 | 0.098 | negative |
| Frisch et al., 2000 | Israelian | 75 | 170 | OCD without TD | DSM-IV | 40bp VNTR | CC | $\chi 2$ | | ns | ns | negative |
| Miguita et al., 2007 | Brazilian | 208 | 865 | OCD±tics | DSM-IV | 30bp VNTR intron 8 | CC | $\chi 2$ | | 0.22 | 0.37 | negative |
| | | 131 | 589 | | | | | | Males | 0.65 | 0.75 | |
| | | 77 | 275 | | | | | | Females | 0.24 | 0.29 | |
| Diaz-Anzaldua et al., 2004 | French-Canadian | 110 | | GTS±OCD | DSM-IV | 40bp VNTR | FB | ETDT | | ns | | negative |
| Tarnok et al., 2007 | Hungarian | 103 | 284 | GTS±OCD | DSM-IV | 40bp VNTR | CC | $\chi 2$ | | 0.797 | 0.859 | negative |
| | | | | | | | FB | TDT | | | 0.188 | |
| Yoon et al., 2007 | Non-Hispanic white | 266 | 236 | GST±ADHD | TSCSG/ DSM-IV | *Dde*I polymorphism | CC | $\chi 2$ | | | **0.015** | Increased heterozygosity |
| | | | | | | 40bp VNTR | CC | $\chi 2$ | | | ns | negative |
| Walitza et al., 2008 | German | 69 | | OCD without GTS | DSM-IV | 40bp VNTR | FB | ETDT | | 0.62 | | negative |
| **Dopamine beta Hydroxylase (DBH) (9q34)** | | | | | | | | | | | | |
| Ozbay et al., 2006 | Canadian/ Turkish | 106 | | GTS, OCD unknown | DSM-IV | *Taq*I (rs2519152) | FB | TDT | | 0.096 | | negative |
| | | | | | | 19bp ins/del | FB | TDT | | 0.907 | | negative |
| | | | | | | $Ca_{(n)}$ repeat | FB | TDT | | ns | | negative |
| Yoon et al., 2007 | Non-Hispanic white | 266 | 236 | GST±ADHD, OCD unknown | TSCSG/ DSM-IV | *Taq*I polymorphism intron 5 | CC | $\chi 2$ | | ns | ns | negative |
| **Acid Phosphatase locus I gene (2p25)** | | | | | | | | | | | | |
| Bottini et al., 2002 | European Caucasian | 184 | 539 | GST±ADHD, OCD unknown | DSM-IIIR | *TaqI* polymorphism non-AA genotype vs other genotypes | CC | | | **0.0004** | **0.00007** | 11 genotype and 1 allele increased in GTS with conduct disorder |
| Yoon et al., 2007 | Non-Hispanic white | 266 | 236 | GST±ADHD, OCD unknown | TSCSG /DSM-IV | *TaqI* polymorphism | CC | $\chi 2$ | | ns | ns | negative |

CC=Case-control, FB=Family based, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, ETDT=Extended Transmission Disequilibrium Test, ns=not significant.

The glutamatergic system

The etiology of OCD may be located in the complex neurotransmitter network of the cortico-striatal-thalamo-cortical tract (Graybiel and Rauch, 2000). Therefore, neurotransmitters other than serotonin and dopamine such as glutamate may play a role in the etiology of OCD (Carlsson, 2000).

The *Glutamate transporter (SLC1A1/EAAD)* gene is located at the susceptibility locus for OCD at chromosome 9p (Hanna et al., 2002). Moreover, 9p monosomy has been described in patients with OCD and comorbid GTS (Taylor et al., 1991). Therefore, the *SLC1A1* gene is an interesting candidate gene for OCD and GTS. However, mutation screening showed no apparently deleterious mutations and no linkage disequilibrium between OCD and the *SLC1A1* gene was detected (Veenstra-VanderWeele et al., 2001). Three recent family-based association studies all found an association of a haplotype and OCD, mainly confined to males (Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007a). A case-contol study found a haplotype associated with OCD in both sexes (Wendland et al., 2009). A fifth study found linkage with another SNP in the 3' region of the *SLC1A1* gene (Shugart et al., 2009). Although the associated haplotypes did not completely overlap, these findings suggest a role for the *SLC1A1* gene in the etiology of OCD, especially in families with male probands. To our knowledge the *SLC1A1* gene has not yet been studied in GTS.

The NMDA *subunit 2B* gene *(GRIN2B* gene*)* encodes the NR2B subunit of the ionotropic glutamate receptor. It is a suitable candidate gene because of its probable involvement in learning processes considering deficits in implicit learning tasks (Deckersbach et al., 2002; Rauch et al., 1997; Remijnse et al., 2006) and failure of striatum activation during implicit learning have been described in OCD (Rauch et al., 1997). An association was found between both the T allele of the T5072G polymorphism and the 5072G-5988T haplotype of the *GRIN2B* gene and OCD (Arnold et al., 2004). The *GRIK2 and GRIK3* gene, encoding ionotropic glutamate receptors (iGluR) were investigated in an association study that used both a population-based and a family-based approach. This study reported an undertransmission of the I867 allele of the GRIK using the family-based approach (Delorme et al., 2004).

The *SAPAP 3* gene is a member of the SAP90/PSD95-associated protein (SAPAP) family of proteins. These proteins form a complex at excitatory glutamatergic synapses. This gene was studied in a family-based association study (Bienvenu et al., 2008). No association was found with OCD. However there was an association between several SNP's in the *SAPAP3* gene and grooming disorders (i.e. pathological skin biting, skin picking and trichotillomania, as well as in patients with OCD and/or trichotillomania (Bienvenu et al., 2008; Zuchner et al., 2009).

Table 11. Association studies investigating glutamate transporter (*SLC1A1*) gene in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Diagnostic criteria | Phenotype | Diagnostic criteria | Design | Test | Subgoup | Allelic p | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SLC1A1 (9p24) | | | | | | | | | | | |
| Dickel et al., 2006* | USA | 66 | | DSMIIIR/ DSM-IV | OCD | rs3780412 | FB | TDT | | **0.04** | association of two SNP's |
| | | | | | | rs3780412 | | | Males | **0.002** | and a haplotype at the 3' |
| | | | | | | rs3780412 | | | Females | 0.87 | region and OCD in males |
| | | | | | | rs301430 | FB | TDT | | **0.03** | |
| Arnold et al., 2006 | Canadian | 157 | | DSM-IV | OCD | rs301979 | FB | FBAT | | 0.49 | Two SNP's and a 3' |
| | | | | | | | | /TDT | Males | 0.07 | haplotype associated with |
| | | | | | | | | | Females | 0.58 | OCD in males |
| | | | | | | rs301434 | | | | **0.0007** | |
| | | | | | | | | | Males | **0.002** | |
| | | | | | | | | | Females | 0.1 | |
| | | | | | | rs301435 | | | | **0.0009** | |
| | | | | | | | | | Males | **0.001** | |
| | | | | | | | | | Females | 0.16 | |
| | | | | | | rs3087879 | | | | **0.006** | |
| | | | | | | | | | Males | **0.002** | |
| | | | | | | | | | Females | 0.31 | |
| Stewart et al., 2007a | USA/French | 66 | | DSM-IV | OCD ±GTS | rs2228622 | FB | FBAT | | 0.19 | 3' haplotype associated |
| | | | | | | | | | Males | **0.045** | with OCD in males |
| | | | | | | | | | Females | 0.3 | |
| | | | | | | rs3780412 | | | | 0.13 | |
| | | | | | | | | | Males | **0.045** | |
| | | | | | | | | | Females | 0.44 | |
| Shugart et al., 2009* | USA | 1006 | | DSM-IV | OCD | rs301443 | FB | PBAT | | **0.000067** | rs301443 associated with |
| | | | | | | | | | Males | **0.00027** | OCD in males |
| | | | | | | | | | Females | 0.076 | |
| Wendland et al., 2009 | USA | 325 | 662 | DSM-IV | OCD | rs3087879 | CC | $\chi 2$ | | 0.12 | haplotype associated with |
| | | | | | | rs301430 | | | | >0.99 | OCD |
| | | | | | | rs7858819 | | | | >0.99 | |

*Studies with partly overlapping samples.
CC=Case-control, FB=Family based, TDT=Transmission disequilibrium test.

Table 12. Association studies investigating genes encoding glutamate receptor subunits in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Diagnostic criteria | Diagnosis | Polymorphism | Design | Test | Allelic p | Genotypic p | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **GRIK2 (6q21)** | | | | | | | | | | | |
| Delorme et al., 2004 | French/German | 156 | 141 | DSM-IV | OCD | rs2227281 | CC | $\chi 2$ | 0.2 | 0.09 | Trend for an association |
| | | | | | | E808E | CC | $\chi 2$ | 0.52 | 0.78 | between the M867I |
| | | | | | | M867I | CC | $\chi 2$ | 0.09 | 0.09 | variant and OCD |
| | | 124 | | | | rs2227281 | FB | TDT | 0.45 | - | |
| | | | | | | E808E | FB | TDT | 0.6 | - | |
| | | | | | | M867I | FB | TDT | **0.03** | - | |
| **GRIK3 (1p34-p33)** | | | | | | | | | | | |
| Delorme et al., 2004 | French/German | 156 | 141 | DSM-IV | OCD | S310A | CC | $\chi 2$ | 0.5 | 0.4 | negative |
| | | 124 | | | | S310A | FB | TDT | 0.57 | - | |
| **GRIN2B (12p12)** | | | | | | | | | | | |
| Arnold et al., 2004 | Canadian | 130 | | DSM-IV | OCD | T5072G | FB | FBAT | **0.014** | - | T5072G variant |
| | | | | | | A5806C | FB | FBAT | 0.44 | - | associated with OCD |
| | | | | | | T5988 | FB | FBAT | **0.04** | - | under non-additive model |

CC=Case-control, FB=Family based, TDT=Transmission Disequilibrium Test.

Enzymes involved in neurotransmitter degradation

*Catechol-O-methyl transferase (COMT)* is involved in the degradation of dopamine, epinephrin and norepinephrin. The *COMT* gene has a functional polymorphism in which a valine residue is substituted for methionine residue at position 158 (*Val158Met).* The *Met158Met* genotype causes three to four times reduced enzyme levels compared to the Val158Val genotype and the activity of the *Val158Met* genotype is in between (Lotta et al., 1995).

An association has been found between the *Met158Met* genotype (Karayiorgou et al., 1997) or the *158Met* allele (Denys et al., 2006a; Pooley et al., 2007) and OCD in males in some studies, whereas an association between the *158Met* allele and OCD was also found in females (Alsobrook et al., 2002b). Other studies found an association between the Val/Met genotype and OCD (Niehaus et al., 2001) or only a tendency for association of the Met/Met genotype and OCD with no evidence for sex difference (Schindler et al., 2000).

Two recent meta-analyses reviewed the association studies of the *Val158Met* polymorphism of the *COMT* gene in OCD. One of these provided insufficient evidence to support an association between the *Val158Met* polymorphism and OCD (Azzam and Mathews, 2003). The other found an association with the *158Met* allele and OCD in males (Pooley et al., 2007).

The expression of the human COMT gene is down-regulated by estrogen, and mediated by Estrogen Responsive Elements (ERE's) in the promoter. An association study investigated a C>T transition near one of these ERE's (ERE6) in OCD (Kinnear et al., 2001) and found no evidence for an association between this polymorphism and OCD.

Three association studies investigated the *COMT Val158Met* polymorphism in GTS with negative result (Cavallini et al., 2000; Lim et al., 2009; Tarnok et al., 2007).

*Monoamine Oxidase A* is involved in the degradation of neurotransmitters such as dopamine and serotonin. Two polymorphisms in the MAO-A gene, T941G and T1460C, are associated with the levels of enzyme activity (Hotamisligil and Breakefield, 1991). Gender specific associations of this gene have been described, but the direction differed. The G941 allele showed a significant association with OCD in males (Karayiorgou et al., 1997), whereas the C1460 allele was associated with OCD in females (Camarena et al., 2001). However, no association between the T1640C polymorphism and OCD was found in a third study (Hemmings et al., 2003).

Table 13. Association studies investigating the *COMT Val158Met* polymorphism in OCD and GTS.

| Study | Population | Phenotype | Cases (n) | Controls (n) | Diagnostic criteria | Design | Test | Subgroup | Allelic p | Genotypic p | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **COMT (22q11.2)** | | | | | | | | | | | |
| Karayiorgou et al., 1997 | USA | OCD±tics | 73 | 148 | DSM-IIIR | CC | $\chi 2$ | | **0.0003** | **0.0001** | Met/Met genotype + Met allele associated with OCD in males |
| | | | 42 | 75 | | | | Males | **0.0002** | **0.0002** | |
| | | | 31 | 73 | | | | Females | 0.9097 | 0.0658 | |
| Ohara et al., 1998b | Japanese | OCD,tics unknown | 17 | 106 | DSM-IV | CC | $\chi 2$ | | ns | - | negative |
| Karayiorgou et al., 1999 | USA | OCD±tics | 110 | | DSM-IV/ RDC/ ICD-9 | FB | TDT | | > 0.05 | - | Met allele associated with OCD in males |
| | | | | | | | TDT | Females | 0.375 | - | |
| | | | | | | | HHRR | Females | 0.3491 | - | |
| | | | | | | | TDT | Males | **0.0079** | - | |
| | | | | | | | HHRR | Males | **0.0146** | | |
| Cavallini et al., 2000 | Italian | GTS±OCD | 52 | 63 | DSM-IV | CC | $\chi 2$ | | 0.704 | 0.878 | negative |
| | | | 30 | 24 | | | | Males | 0.773 | 0.833 | |
| | | | 22 | 39 | | | | Females | 0.308 | 0.524 | |
| Schindler et al, 2000 | USA/Canadian | OCD tics unknown | 72 | | DSM-IV | FB | TDT | | 0.54 | | Trend for homozygosity (p=0.017) |
| | | | | | | | | Males | 0.86 | | |
| | | | | | | | | Females | 0.51 | | |
| | | | | | | | HHRR | | 0.48 | 0.056 | |
| | | | | | | | | Males | 0.35 | 0.64 | |
| | | | | | | | | Females | 0.24 | 0.17 | |
| Niehaus et al., 2001 | South African | OCD±tics | 54 | 54 | DSM-IV | CC | $\chi 2$ | | | **0.0017** | Increased frequency of heterozygosity |
| | | | 26 | 26 | | | | Males | | **0.043** | |
| | | | 28 | 28 | | | | Females | | **0.029** | |

CC=Case-control FB=Family based, TDT=Transmission Disequilibrium Test, HHRR=Haplotype-based Haplotype Relative Risk, ns=not significant

Table 14. Association studies investigating the *COMT* gene in OCD and GTS.

| Study | Population | Diagnosis | Cases (n) | Controls (n) | Diagnostic Criteria | Polymorphism | Design | Test | Subgroup | Allelic p | Genotypic p | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **COMT (22q11.2)** | | | | | | | | | | | | |
| Alsobrook et al., 2002b | Israeli/French/ USA | OCD, tics unknown | 56 | | DSM-IIIR | Val158Met | FB | HHRR | | 0.174 | 0.430 | Met allele associated with OCD in females |
| | | | 26 | | | | | | Males | 0.422 | | |
| | | | 30 | | | | | | Females | **0.048** | | |
| | | | 56 | | | | | TDT | | 0.365 | | |
| | | | 26 | | | | | | Males | 0.752 | | |
| | | | 30 | | | | | | Females | 0.105 | | |
| Erdal et al., 2003 | Turkish | OCD±tics | 59 | 114 | DSM-IV | Val158Met | CC | $\chi 2$ | | | 0.82 | Negative |
| Denys et al., 2006a | Dutch | OCD, tics unknown | 155 | 150 | DSM-IV | Val158Met | CC | $\chi 2$ | | 0.643 | 0.779 | Met allele associated with OCD in males |
| | | | 56 | 79 | | | | | Males | **0.035** | 0.100 | |
| | | | 99 | 71 | | | | | Females | 0.227 | 0.440 | |
| Meira-Lima et al., 2004 | Brazilian | OCD, tics unknown | 79 | 202 | DSM-IV | Val158Met | CC | $\chi 2$ | | 0.4 | 0.5 | negative |
| Poyurovsky et al., 2005 | Israeli | OCD | 79 | 171 | DSM-IV | Val158Met | CC | $\chi 2$ | | ns | ns | Met allele associated with OCD in males |
| | | | 34 | 75 | | | | | Males | ns | **0.04** | |
| | | | 45 | 96 | | | | | Females | ns | ns | |
| Pooley et al., 2006 | United Kingdom | OCD | 87 | | DSM-IV | Val158Met | CC | $\chi 2$ | | 0.069 | | Met allele associated with OCD in males |
| | | | 30 | | | | | | Males | **0.026** | | |
| | | | 57 | 190 | | | | | Females | 0.565 | | |
| Tarnok et al., 2007 | Hungarian | GTS | 103 | 284 | DSM-IV | Val158Met | CC | | | 0.488 | 0.595 | negative |
| | | | | | | | TDT | | | 0.541 | | |
| Walitza et al., 2008 | German | OCD without GTS | 69 | | DSM-IV | Val158Met | TDT | | | 0.66 | | negative |
| Lim et al., 2009 | Korean | GTS | 72 | 100 | DSM-IV | Val158Met | CC | $\chi 2$ | | 0.92 | 0.62 | negative |
| Kinnear et al., 2001 | South African | OCD, tics unknown | 48 | 48 | DSM-IV | C>T near ERE6 promoter | CC | $\chi 2$ | | NA | 0.93 | negative |

CC=Case-control, FB=Family based, TDT=Transmission Disequilibrium Test, HHRR=Haplotype-based Haplotype Relative Risk, ns=not significant

Table 15. Association studies investigating the *MAO-A* gene in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Phenotype | Diagnostic criteria | Polymorphism | Design | Test | Subgroup | Allelic p | Genotypic p | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MAO-A (Xp11.23)** | | | | | | | | | | | | |
| Karayiorgou et al., 1999 | USA | 110 | | OCD±tics | DSM-IV | T941G | FB | TDT | Males | **0.0186** | | G allele associated with OCD in males |
| | | | | | | | | TDT | Females | 0.1025 | | |
| | | | | | | | | HHR | Males | **0.0129** | | G allele associated with OCD in males |
| | | | | | | | | | Females | 0.1002 | | |
| Camarena et al., 2001 | Mexican | 122 | | OCD, tics unknown | DSM-IV | T1060C | FB | HRR | Males<br>Females | | | T allele risk factor in females |
| | | 122 | 24 | | | | CC | $\chi 2$ | | **0.002** | | C allele associated with OCD in males |
| | | 63 | 60 | | | | | $\chi 2$ | Males | **0.0053** | | |
| Hemmings et al., 2003 | South African | 71 | 129 | OCD, tics unknown | DSM-IV | T1060C | CC | $\chi 2$ | Males | 0.61 | | negative |
| | | | | | | | | | Females | 0.55 | 0.69 | |
| Diaz-Anzaldua et al., 2004 | French-Canadian | 110 | | GTS±OCD | DSM-IV | 30bp VNTR promoter *Fnu*4H1 *Eco*RV | FB | TDT/ ETDT | | **0.0076** | | high activity allele promoter VNTR and high activity high activity haplotype |

CC=Case-control, FB=Family based, TDT=Transmission Disequilibrium Test, ETDT=Extended Transmission Disequilibrium Test, HHRR=Haplotype Relative Risk, ns= not significant.

Table 16. Association studies investigatine the *GABBR1* gene, the *MOR* gene, the *MOG* gene, the *OLIG2* gene and the *SGCE* gene in OCD and GTS.

| Study | Population | Cases (n) | Controls (n) | Diagnostic criteria | Diagnosis | Polymorphism studied | Design | Test used | Subgroup | Allelic (p) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **GABBR1 (6p21.3)** | | | | | | | | | | | |
| Zai et al., 2005a | Canadian | 159 | | DSM-IV | OCD±tics | A-7265G | FB | TDT/STDT | | **0.006** | A allele overtransmitted |
| | | | | | | C10497G | FB | TDT/STDT | | 0.163 | negative |
| | | | | | | A33795G | FB | TDT/STDT | | 0.442 | negative |
| | | | | | | T1473C | FB | TDT/STDT | | **0.032** | A allele overtransmitted |
| | | | | | | T1977C | FB | TDT/STDT | | 0.319 | negative |
| **MOR (6q24-25)** | | | | | | | | | | | |
| Urraca et al., 2004 | Mexican | 51 | | DSM-IV | OCD±tics | C17T | FB | TDT | | NA | negative |
| | | | | | | A118G | FB | TDT | | > 0.1 | |
| | | | | | | | | | Comorbid tics | 0.065 | |
| **MOG (6p21.1-p22)** | | | | | | | | | | | |
| Zai et al., 2004 | Canadian | 160 | | DSM-IV | OCD, tics unknown | MOG2 | FB | TDT | | 0.061 | Negative |
| | | | | | | MOG4 | FB | TDT | | **0.022** | Overtransmission allele 2 |
| | | | | | | C1334T | FB | TDT | | 0.611 | negative |
| | | | | | | C10991T | FB | TDT | | 0.696 | negative |
| Huang et al., 2004 | Chinese | 197 | | DSM-IIIR | GTS/CMT, OCD unknown | MOGa | FB | TDT | | 0.409 | negative |
| | | | | | | MOGb | FB | TDT | | 0.254 | negative |
| | | | | | | MOGc | FB | TDT | | 0.0615 | negative |
| **OLIG2 (21q22.11)** | | | | | | | | | | | |
| Stewart et al., 2007b | USA/ French | 91 | | DSM-IV | OCD±TD | rs762178 | FB | FBAT | OCD without TD | **<0.001** | Overtransmission G allele |
| | | | | | | rs1059004 | | | OCD without TD | **0.005** | Overtransmission A allele |
| | | | | | | Rs9653711 | | | OCD without TD | **0.004** | Overtransmission G allele |
| **Epsilon-sarcoglycan (7q21)** | | | | | | | | | | | |
| De Carvalho Aguiar et al., 2004 | USA | 32 | 60 | DSM-IV | GTS+OCD | Dinucleotide repeat exon 3 | CC | $\chi 2$ | GTS + OCD | 0.83 | negative |

CC=Case-control, FB=Family based, TD=Tic Disorder, TDT=Transmission Disequilibrium Test, STDT=Sibship Transmission Disequilibrium Test

Table 17. Association studies investigating the *BDNF* gene, the glutamate transporter gene and the *APOE* gene in OCD and GTS.

| Study | Population | No of cases | No of controls | Diagnostic criteria | Phenotype | Polymorphisms | Design | Test | Allelic p | Genotypic p | Result |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BDNF (11p13-14)** | | | | | | | | | | | |
| Hall et al., 2003 | USA | 164 | | DSM-IV | OCD±GTS/tics | Val66Met | FB | TDT | **0.0005** | - | Undetransmission of |
| | | | | | | rs988748 | FB | TDT | **0.0005** | - | the Met allele and |
| | | | | | | rs2049046 | FB | TDT | **0.012** | - | haplotype with Met |
| | | | | | | C11757G | FB | TDT | **0.0025** | - | allele |
| Mössner et al., 2005 | German | 67 | | DSM-IV | OCD without GTS | Val66Met | FB | TDT | 0.62 | | negative |
| Zai et al., 2005b | Canadian | 152 | | DSM-IV | OCD, tics unknown | Val166Met | FB | FBAT | 0.587 | - | negative |
| | | | | | | $(GT)_n$ | FB | FBAT | 0.654 | - | negative |
| Klaffke et al., 2006 | German | 88 | | DSM-IIIR | GTS±OCD | Val166Met | FB | TDT | 0.414 | - | negative |
| Dickel et al., 2007 | | 54 | | DSM-IIIR | OCD± GTS | Val166Met | FB | TDT | 1.00 | - | negative |
| Wendland et al., 2007 | Caucasian | 347/295 | 657 | DSM-IV | OCD±tics | Val166Met | CC | $\chi 2$ | ns | | negative |
| Hemmings et al., 2008 | Afrikaner | 112 | 140 | DSM-IV | OCD±tics | Val166Met | CC | LR | | | Met allele associated with OCD in males |
| Alonso et al., 2008a | Spanish | 115 | | DSM-IV | OCD±tics | Val166Met | CC | LR | | | negative |
| **SLC6A2 (16q12.2)** | | | | | | | | | | | |
| Rippel et al., 2006 | Non-Hispanic white | 225 | 120 | TSCG | GTS | T-182C | CC | NA | ns | ns | negative |
| | | | | | | G1287A | CC | NA | ns | ns | negative |
| **Tumor Necrosis Factor Alpha (TNFA) 6p21.3-21.1** | | | | | | | | | | | |
| Zai et al., 2006 | Canadian | 127 | | DSM-IV | OCD | -308G/A | FB | FBAT | 0.291 | | negative |
| Houni et al., 2008 | Brazilian | 111 | 250 | DSM-IV | OCD±TD | -308G/A | CC | $\chi 2$ | **0.007** | **p<0.012** | A allele associated with OCD |
| | | | | | | -238G/A | CC | $\chi 2$ | **0.0005** | **p<0.002** | A allele associated with OCD |
| **APOE (19q13.12)** | | | | | | | | | | | |
| Nicolini et al., 2001 | Mexican | 49 | ? | DSM-IV | OCD | APO ε4 allele | CC | $\chi 2$ | | 0.17 | negative |

CC=Case-control, FB=Family based, TDT=Transmission Disequilibrium Test, LR=logistic regression, ns= not significant

Epsilon sarcoglycan *(SGCE)* gene

The *epsilon sarcoglycan gene (SGCE* gene*)* has been investigated in GTS and OCD since both symptomatic and asymptomatic carriers of mutations in the *SGCE* gene in some families with Myoclonus-Dystonia (M-D) show an increased frequency of OCD (Hess et al., 2007; Saunders-Pullman et al., 2002). No mutations were found in 32 GTS patients with comorbid OCD and a dinucleotide GT repeat in exon 3 of the *SGCE* gene showed no evidence for an association with OCD and comorbid GTS (de Carvalho Aguiar et al., 2004). There were also no clearly pathogenic mutations in a group of 83 sporadic GTS patients (Asmus et al., 2005b). Recently, linkage with known M-D loci was excluded in a family with autosomal dominant M-D, GTS and OCD suggesting the presence of another susceptibility locus for M-D, GTS and/or OCD in this family. (Orth et al., 2007).

Brain Derived Neural Growth Factor *(BDNF)*

Since it has been hypothesized that OCD might be a neurodevelopmental disorder (Rosenberg and Keshavan, 1998), genes involved in neuronal development have been studied in OCD. The *BDNF* gene encodes a growth factor involved in cell survival, differentiation and cell death. The *Val66Met* polymorphism in this gene causes a reduced depolarization-induced BDNF secretion (Egan et al., 2003). In a family-based association undertransmission of the *BDNF 66Met* allele and a haplotype containing this allele was reported (Hall et al., 2003). When the sample was divided according to age at onset, the association was restricted to early-onset OCD (<18 years of age) (Hall et al., 2003). Three other family based association studies did not replicate this finding (Dickel et al., 2007; Mössner et al., 2005; Zai et al., 2005b). One case-control study found an association between the *BDNF 66Met* allele and OCD (Hemmings et al., 2008), whereas two other case-control studies found no evidence of association (Alonso et al., 2008a; Wendland et al., 2007). For GTS, only one family-based association study with the *BDNF Val66Met* polymorphism has been performed with negative result (Klaffke et al., 2006). In one of the *BDNF* association studies, the gene encoding the *BDNF* receptor, the Neurotrophic Tyrosine Kinase Receptor 2 *(NTRK2)* gene was also investigated and a haplotype protective for OCD was found (Wendland et al., 2007).

Genes related to the auto-immune/PANDAS hypothesis of OCD

*Myelin Oligodendrocyte glycoprotein (MOG)* is located in the myelin sheath and has been implicated in demyelinating autoimmune diseases (Johns and Bernard, 1997). The *MOG* gene has been investigated in OCD since some OCD cases are preceded by

group A β-hemolytic streptococcal infection and mediated by anti-neural antibodies (Snider and Swedo, 2004). An association was found between OCD and allele 2 of the MOG4 polymorphism and the C1334/MOG2 allele 13/T100991/MOG4 allele 2 haplotype (Zai et al., 2004), but this association has yet to be replicated in other studies. Moreover, it is not clear whether the actual putative susceptibility risk site is at this locus in the MOG gene or in a HLA-related site in linkage disequilibrium with this MOG marker which itself has not been investigated in OCD or GTS (Zai et al., 2004). The investigation of three polymorphisms in the *MOG* gene in parent-GTS offspring trio's, did not yield evidence for an association with GTS (Huang et al., 2004). An association analysis of two polymorphisms (G310R and S138G) in the interleukin-10 receptor of 77 GTS patients gave also negative results (Kindler et al., 2008).

*SLITRK1/ROBO* pathway

Slit proteins are expressed in the central nervous system and play a role in neuronal outgrowth and axonal guidance (Aruga and Mikoshiba, 2003; Aruga et al., 2003). Roundabout (Robo) proteins are receptors for the slit proteins.

Recently, the identification of a patient with a de novo chromosome 13 inversion, inv(q31.1;q33.1) prompted researchers to investigate the Slit and Trk-like family member 1 (*SLITRK1)* gene in this region in 174 GTS patients (Abelson et al., 2005). In one patient a frameshift mutation was detected, in two other patients a noncoding sequence variant (var321) in a putative miRNA binding site the 3' untranslated regions was detected. Association studies of over 1610 patients with GTS/chronic tics and 322 OCD patients did not show an association between these variants and GTS or OCD (Keen-Kim et al., 2006; Miranda et al., 2008b; Scharf et al., 2008; Wendland et al., 2006). Two reports about large families with GTS and OCD found the frameshift mutation, the var321 nor other apparent pathogenic mutation in the *SLITRK1* gene (Fabbrini et al., 2007; Verkerk et al., 2006). Mutation screening in 334 patients did not show the var321 (Chou et al., 2007; Deng et al., 2006; Zimprich et al., 2008). However, the var321 variant was found in controls (Keen-Kim et al., 2006; Wendland et al., 2006). In an association study of the *SLITRK1* var321 in a Costa Rican population and an Ashkenazi Jewish population, the variant was only found in the Ashkenazi Jewish population and was transmitted only in three of five cases. These findings suggest that the association proposed by Abelson, may be caused by hidden stratification between cases and controls. Moreover, a family-based association study of two other genes in the Robo-Slit pathway, the roundabout 3 (*ROBO3)* and roundabout 4 *(ROBO4)* gene, did not provide evidence for linkage a role for these genes in the etiology of GTS (Miranda et al., 2008a). However, two rare mutations were found in two families with

Trichotillomania (Zuchner et al., 2006). In conclusion, variants in the *SLITRK1* gene seem to account for only rare cases of GTS, but may have a possible role in OCD spectrum disorders such as trichotillomania.

Other genes

Other genes investigated in association studies for OCD and/or GTS are the Oligodendrocyte lineage transcription factor 2 *(OLIG2)* gene, the Acid phosphatase locus1 (*ACP1*) gene, the μ opioid receptor *(MOR)* gene, the Apolipoprotein E *(APOE)* gene, the Noreopinephrine transporter gene (*SLC6A2)* and the gamma-aminobutyric acid type B receptor 1 gene (*GABBR1*). The associations between three SNP's in the *OLIG2* gene and OCD without GTS, the functional G118A polymorphism in the *MOR* gene and OCD with comorbid tics, the association between the.*ACP1* gene and GTS, and the association between the *GABBR1* gene and OCD have not yet been replicated (Bottini et al., 2002; Stewart et al., 2007; Urraca et al., 2004; Zai et al., 2005a). The Apolipoprotein E *(APOE)* gene and the *SLC6A2* gene showed no evidence for association with OCD and GTS respectively (Nicolini et al., 2001; Rippel et al., 2006).

Genome-wide candidate-gene association study

In addition to studies investigating single genes or genes in a single pathway described here-above, a recent study investigated SNP's in 306 genes involved in neurotransmission and neurodevelopment in seven neuropsychiatric disorders including OCD and two control groups including >3000 samples (Gratacos et al., 2008). A SNP in the bradikinin receptor B2 (*BDKRB2*) gene was associated with OCD testing a dominant model using one of the two control groups, after correcting for multiple testing. Bradikinin causes calcium-dependent glutamate release from cortical astrocytes and is an interesting candidate gene in OCD in light of the putative role of glutamate in OCD (Parpura et al., 1994).

**Future prospects for the molecular genetic studies in GTS and OCD**

Except for linkage at chromosome 9p for OCD, more specifically the SLC1A1 gene in male patients, none of the positive findings in the molecular genetic studies of OCD and GTS have been extensively replicated. Thus despite the tremendous effort, the search for susceptibility genes and susceptibility loci for GTS and OCD has been hampered by inconsistent results. Two categories of factors probably contribute to these inconsistent results:
1) Symptomatic and genetic heterogeneity of OCD and 2) Methodological limitations

Phenotypic heterogeneity of OCD and GTS
The heterogeneity of OCD and GTS may contribute to the inconsistent results of the molecular genetic studies described here-above. Therefore, the identification of more homogeneous OCD subtypes according to symptoms, gender, age of onset and family may aid in the search for susceptibility genes of these disorders.
*Symptomatic heterogeneity of OCD*
The Yale-Brown Obsessive-compulsive symptom checklist (YBOCS-CL) (Goodman et al., 1989a, 1989b) is a widely used instrument to investigate obsessive-compulsive symptoms. Several studies performed factor or cluster analysis on the YBOCS-CL to identify symptom dimensions (see Bloch et al, 2008a and Mataix-Cols et al., 2005 for recent reviews of these studies). Four highly replicable symptom dimensions are identified in these studies: 1) aggressive and sexual obsessions and checking compulsions, 2) Symmetry/ordering obsessions and arranging and counting compulsions, 3) Contamination obsessions with cleaning compulsions and 4) hoarding obsessions and compulsions.
Evidence suggests that these symptom dimensions represent clinically relevant subtypes. For example, patients with hoarding obsessions and compulsions improved less on both cognitive behavior therapy (Abramowitz et al., 2003) and SSRI treatment (Mataix-Cols et al., 1999) and have an increased dropout rate from behavior therapy (Mataix-Cols et al., 2002a). Furthermore, it has been shown that washing, checking and hoarding symptom dimensions in OCD are associated with activation in specific brain areas (Mataix-Cols et al., 2004; van den Heuvel et al., 2004), suggesting that distinct brain regions mediate these symptom dimensions.
The symptom dimensions may also represent genetically distinct subtypes. Segregation analyses in OCD and GTS patients show that symptom dimensions are transmitted within families with possible differential transmission (Alsobrook et al., 1999; Leckman et al., 2003). Since the hoarding and aggressive/sexual obsessions and

checking dimensions were associated with an increased risk of OCD or subclinical OCD in first-degree relatives, these symptom dimensions may represent a more familial subform of OCD. Some studies already used category-based symptom dimensions in molecular genetic studies of OCD. Three studies investigated the 5HTTPR polymorphism. In the first study, the LL genotype scored higher on a symptom dimension compared to patients with LS or SS genotypes (Cavallini et al., 2002), a second study showed that the frequencies of the SS genotype and S allele were associated a symptom dimension with symmetry obsessions, ordering, arranging and repeating compulsions (Hasler et al., 2006) whereas a third study found that patients with the SS genotype scored lower on a symptom dimension with somatic and religious obsessions (Kim et al., 2005). Two studies that investigated symptom dimensions in association studies of the *BDNF Val66Met* polymorphism had negative results (Alonso et al., 2008a; Wendland et al., 2007).

Hoarding was found to be associated to a SNP in the Neurotrophic Tyrosine Kinase Receptor type 3 gene (Alonso et al., 2008b). Two genome wide linkage studies used hoarding as a phenotype. One of these studies was performed in sib pairs affected with GTS and showed evidence suggestive for linkage of hoarding to chromosome 4q34-35, 5q35.2-35.3 and 17q25. These regions may coincide with regions that previously showed evidence for linkage in linkage studies for GTS (Paschou et al., 2004; TSAICG, 1999; Verkerk et al., 2006). The second study in multiplex OCD families in which probands with GTS were excluded, found evidence suggestive for linkage at chromosome 14q (Samuels et al., 2007). Interestingly, it was subsequently found that conditioning linkage at this 14 region on a marker adjacent to the glutamate transporter gene at chromosome 9p decreased the size of the 14q linkage region suggesting an interaction between these regions at chromosome 14q and a region at chromosome 9p near the glutamate transporter gene (Liang et al., 2008).

Most factor-analytic studies addressing the symptomatic heterogeneity of OCD have limitations. For example, most studies have limited sample sizes and analyze YBOCS-CL symptom categories rather than individual items. Future studies addressing these limitations are warranted.

Even more important is to determine the validity of the use of these symptom dimensions in genetic studies, the heritability of the symptom dimensions.

*Symptomatic heterogeneity of GTS*

A recent cluster analysis of tic symptoms in two samples grouped GTS patients in two clusters according to their tics (Mathews et al., 2007a). One of these clusters consisted of patients with predominantly simple tics; whereas patients in the other cluster were

characterized by multiple complex tics and had more obsessive-compulsive symptoms. Another study performed cluster analysis and used the resulting clusters for subsequent factor analysis (Alsobrook and Pauls, 2002). This gave four factors: 1) aggressive phenomena 2) purely motor and phonic tic symptoms 3) compulsive phenomena 4) tapping and the absence of grunting. High intraclass correlations were found within families for factor 1, 2 and 4, suggesting these may comprise heritable components of GTS. Factor 3 was associated with OCD in relatives. A similar approach in a large multigenerational family yielded 3 factors: 1) predominantly 'pure' tics 2) predominantly attention deficit hyperactivity disorder and aggressive behaviors and 3) predominantly 'depression-anxiety-obsessional symptoms and self-injurious behaviors (Robertson and Cavanna, 2007). To our knowledge, no molecular genetic studies using symptom dimensions in GTS as phenotypes have been published yet.

In GTS the identification of symptom dimensions is at an early stage.

*Heterogeneity by gender*

There is evidence for clinical and genetic heterogeneity by sex. For example, male patients have an earlier age at onset of the disease and more often suffer from comorbid tics (Lochner et al., 2004). It is unclear whether these differences between males and females are due to genetic influences, hormonal influences or both. However, there is ample evidence for a genetic heterogeneity by sex. Segregation analysis suggests also genetic heterogeneity by sex (Nestadt et al., 2000a) and shows a higher penetrance for the genetic susceptibility gene(s) in females compared to males (Cavallini et al., 1999). Furthermore, in association studies sex-specific associations were found for 5HT1Dβ in Afrikaner males (Lochner et al., 2004), the 5HT2A gene in females (Enoch et al., 2001; Walitza et al., 2002) and the *DRD2* gene (Denys et al., 2006a) and the *BDNF* gene (Hemmings et al., 2008). Gender-specific associations have also been reported for the *COMT* gene (Alsobrook et al., 2002b; Denys et al., 2006a; Karayiorgou et al., 1997, 1999; Pooley et al., 2007; Poyurovsky et al., 2005) and the *MAO-A*-gene (Camarena et al., 2001; Karayiorgou et al., 1999; Lochner et al., 2004), but the direction of these gender-specific associations differed. Thus, although there seems to be a genetic basis for the heterogeneity by sex for OCD, the nature of this genetic basis is yet poorly understood.

*Age of onset*

Age of onset of OCD shows a bimodal distribution with two peaks: the first around 11 years and the second around 24 (Delorme et al., 2005). Since early-onset OCD shows a higher familiarity than late-onset OCD (Pauls et al., 1995) age at onset has been used to form more homogeneous subforms of OCD (Hall et al., 2003).

*Family history*

Patients with a positive family history for OCD have more ordering compulsions and anxiety disorders compared to patients with a negative family history of OCD (Hanna et al., 2005a). OCD patients with a positive family history for tics had a lower age at onset and possibly were more often male compared to patients with a negative family history of tics

*Methodological limitations*

Sample size

One of the reasons of the inconsistent results or the molecular genetic studies in OCD and GTS may be that most studies may be underpowered due to limited sample sizes. Large, multicenter studies are necessary in order to increase sample sizes and power. For GTS the Tourette syndrome International Consortium for Genetics has been founded. Recently, a multicenter collaborative genetics study for OCD has been started in the USA (Samuels et al., 2006). Alternatively, a meta-analytic approach can be used to increase power to detect association.

Gene-environment interactions

Since twin studies show both an environmental and a genetic component in the etiology of OCD, investigating gene-environment interactions may be a useful approach. However, to our knowledge, no gene-environment studies have been published for OCD and GTS. Several studies have investigated the role of life events in the etiology of OCD (Gothelf et al., 2004; Khanna et al., 1988; McKeon et al., 1984). Although the results are inconsistent, these studies generally support a role for life events in the etiology of OCD. Therefore, there might be an interaction between genetic polymorphisms and life events in the etiology of OCD and interactions between polymorphism in candidate genes and life events should be studied in OCD. In contrast to OCD, the number and impact of life events do not seem to be related to the onset of Tourette syndrome (Horesh et al., 2008). Other potentially interesting environmental factors are head trauma (Grados et al., 2008), perinatal adverse events (Santangelo et al., 1994; Vasconcelos et al., 2007) and Beta Hemolytic Streptococcal infection (Moretti et al., 2008; Snider and Swedo, 2004).

Limited genes and limited number of SNP's per gene are studied.

Another limitation of the molecular genetic studies of OCD and GTS performed to date is that most of the studies investigated only one gene or a few genes in a pathway. Moreover, many of these studies investigated only one polymorphism per gene. The common disease common variant (CD/CV) hypothesis proposes that genetic risk factors for common diseases are often caused by common interacting alleles with relatively high frequencies (Hemminki et al., 2008). These alleles typically confer a low increase in risk for the disease and explain only a low fraction of the familial risk. However, due to their high frequency they are associated with a large proportion of disease occurrence (Hemminki et al., 2008).

If the heritability of GTS and OCD could be explained by the CD/CV hypothesis, multiple genes are probably involved. Therefore, multiple genes in pathways known to be involved in OCD and/or GTS should be studied. Moreover, enough SNP's should be genotyped to cover the genes studied. The CD/CV poses that common diseases are caused by common variant with modest effect. Association studies are more powerful than linkage studies to identify variants with modest effect on disease risk (Risch and Merikangas, 1996). Due to developments in genotyping technology and reduced genotyping costs it is currently feasible to genotype multiple SNP's at a time using microarrays. This caused a shift towards Genome wide association studies (GWAS), in which a dense map of SNP's covering the whole genome are used to compare allele frequencies between cases and controls in molecular genetic studies of common diseases (Kruglyak, 2008; Plomin and Davis, 2009). An advantage of genome wide approaches such as GWAS is that the entire genome is covered and that is not dependent on any hypotheses concerning genes or loci involved. Since non-coding DNA plays an important role in the expression of protein-coding DNA, an important advantage of a genome wide approach is that disease associated variants in these regions can also be detected (Plomin and Davis, 2009). GWAS thus seems to be a useful approach and both for OCD and GTS GWAS are currently performed.

The shift towards GWAS raises the question whether or not there will still be a need for candidate studies. Most commercially available genotyping arrays are based on HapMap tagging SNPs. These arrays provide excellent coverage of HapMap SNPs, but considerable lower coverage of and power to detect association with the actual common variation (Bhangale et al., 2008). Therefore, there will still be a need for candidate gene studies, especially when looking at SNP's with low allele frequencies and for the follow up of regions found in GWAS.

An example of study investigating multiple genes in multiple possibly relevant pathways is a recent association study in which 748 SNP's at 306 candidate genes were studied in 7 disorders including OCD (Gratacos et al., 2008).

Strategies to deal with possible population stratification

A large part of the studies use a case-control design and may be biased by population stratification. Population stratification may have caused false positive results which may one of the reasons for non-replications. However, genes and polymorphism may play a different role in the etiology of OCD and/or GTS in different populations. This may result in non-replication of true positive associations. Careful matching of cases and controls can prevent population stratification. Alternatively, family-based approach can be used. A disadvantage of family-based approaches is the lower power compared to case-control studies (Cordell and Clayton, 2005). Moreover, more subjects have to be genotyped in family-based association studies.

Therefore, case-control studies are more cost-effective. Moreover, genomic control or structured association methods which have been developed to control for population stratification (Pritchard and Donnelly, 2001). In these methods, genotypes of several SNP's are used to correct for potential population stratification. To our knowledge, none of the case-control studies performed in GTS has used these methods, whereas only one study in OCD has used the genomic control method (Gratacos et al., 2008). Future association studies should perform careful matching between controls, use genomic control or structured association methods or family-based approaches.

**Aims and outline of this thesis**

The aims of this thesis are to refine and extend the present knowledge about the symptomatic heterogeneity in OCD and GTS and to use this knowledge to investigate the influence of two important candidate genes (the *COMT* gene and the *BDNF* gene) on the phenotypic expression of OCD and GTS.

In Chapter two, an attempt is made to refine and extend the symptom dimensions identified by classical category-based factor analysis of OCD symptoms based on the YBOCS-CL categories. Item-level factor analysis on YBOCS-CL data of a large cohort of OCD patients is performed in a multicenter international study. Some methodological limitations of previous factor analytic studies are addressed and the results are compared to classical category-based factor analysis. In addition, heritability of the symptom dimensions obtained is investigated. In addition, the relation of these symptom dimensions with other clinical characteristics such as gender, age of onset and family history for OCD are investigated.

In chapter three, Latent Class analysis is used to define classes of patients based on OCD symptomatology. The correlation of class-membership with clinical characteristics such as age of onset, sex and tics are investigated.

In chapter four, item-level factor analysis on the YBOCS-CL is performed and the resulting symptom dimensions as well as other phenotypes are analyzed in an association study of the *BDNF Val66Met* polymorphism.

In chapter five, the refined symptom dimensions identified by item-level factor analysis on the YBOCS-CL are used as phenotypes in an association analysis of the *COMT Val158Met* polymorphism. In addition, OCD as a dichotomous trait and additional phenotypes such as age of onset, severity and family history of obsessive compulsive symptoms are analyzed in an association study of this polymorphism. Sex-specific associations are also investigated.

In chapter six, the symptomatic heterogeneity of GTS is investigated by performing item-level factor analysis on Yale Tic Symptom checklist data. Subsequently, an association study using the resulting factors and other phenotypes is performed for the *BDNF Val66Met* and *COMT Val158* polymorphism in a cohort of GTS patients.

Chapter seven describes a mutation screening of the *SGCE* gene in a cohort of mainly familial cases of GTS, Chronic Motor Tic disorder (CMT) and/or OCD.

# Part 1

# Studies of the symptomatic heterogeneity of obsessive compulsive disorder

# Heritability and clinical correlates of the symptom dimensions of OCD

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*Submitted.*

**Abstract**

*To advance our understanding of obsessive-compulsive disorder (OCD), factor analyses of the Yale-Brown Obsessive Compulsive Scale symptom checklist (YBOCS-CL) items have been performed. Only limited data concerning the heritability of factor-based symptoms are available.*

*We performed factor analysis of individual YBOCS items in 1224 OCD subjects and heritability analyses of the resulting factors in a subgroup of OCD subjects and their relatives. Besides a one factor model, a five factor model consisting of 1) taboo, 2) contamination/cleaning, 3) doubts, 4) miscellaneous superstitions/rituals and 5) symmetry/hoarding showed the best fit. All factors except factor 4 were clearly heritable, as were OC symptom severity and total symptom count. The taboo factor was associated with male gender. No symptom dimensions were associated with family history of OCD. These results suggest that a common genetic factor underlies OCD susceptibility, and additional unique genetic factors contributing to four of the five symptom factors.*

## Introduction

Obsessive-compulsive disorder (OCD) is a neuropsychiatric condition that affects 1-2% of the population world-wide and is characterized by intrusive, recurrent thoughts, feelings, and ideas (obsessions) and repetitive actions, often aimed at reducing tension or anxiety accompanying the obsessive thoughts (compulsions) (American Psychiatric Association, 1994; Fontenelle and Hasler 2008).

OCD is phenomenologically and etiologically heterogeneous (Mataix-Cols et al., 2007). The heterogeneity of symptoms in OCD may contribute to the difficulty in finding susceptibility genes, involved in certain aspects of the disorder. It may also dilute findings from other etiological, clinical, and treatment studies. One approach for minimizing the heterogeneity among individuals with OCD that has received some support from neuroimaging, treatment, and genetic studies is to use symptom-based rather than disorder- or syndrome-based constructs, with the idea that underlying symptom dimensions reflect more etiological homogeneity than a global OCD diagnosis (Mataix-Cols et al., 2005).

Obsessive-compulsive (OC) symptomatology is most commonly measured with the Yale-Brown Obsessive Compulsive Scale (YBOCS), which includes an assessment of OC symptom severity and a symptom checklist (YBOCS-CL) containing 45 obsessions and 29 compulsions within 15 predefined symptom categories (Goodman et al., 1989a, 1989b). To reduce the phenomenological heterogeneity of OCD, several factor analyses have been performed using the YBOCS-CL (Baer, 1994; Cavallini et al., 2002; Cullen et al., 2007; Delorme et al., 2006a; Denys et al., 2004b; Feinstein et al., 2003; Girishchandra and Khanna, 2001; Hasler et al., 2005, 2006, 2007; Kim et al., 2005; Leckman et al., 1997; Mataix-Cols et al., 1999, 2005, 2008; McKay et al., 2006; Pinto et al., 2008; Stein et al., 2007, 2008; Stewart et al., 2007c, 2008; Wu et al., 2007). The majority of these studies, which have resulted into three or four main symptom groups, have factor analyzed 15 clinically predefined symptom categories rather than using the individual items, primarily because of methodological constraints and concerns about small sample sizes. A recent symptom category-based meta-analysis of twenty-one factor analyses identified four OC symptom dimensions: 1) symmetry obsessions, counting, ordering and arranging compulsions; 2) obsessions and checking (aggressive, sexual, religious and somatic obsessions and related checking compulsions); 3) contamination/cleaning, and 4) hoarding (Bloch et al., 2008a).

Although straightforward to conduct, the categorical approach has the drawback that individual symptoms grouped in the predefined YBOCS symptom categories (designed to fit a presupposed theoretical model) may not cluster together if assessed separately. In addition, obsessions and compulsions categorized as "miscellaneous" in the YBOCS-CL are not usually included in category-driven analyses, limiting the available items for analysis. Therefore, symptom dimensions resulting from category-driven analyses might be biased (Denys et al., 2004a; Feinstein et al., 2003).

To address this limitation, eight studies (table 1) have been published on exploratory item-level factor analysis using the individual items from the YBOCS-CL (Denys et al., 2004a; Feinstein et al., 2003; Girishchandra and Khanna, 2001; Hantouche and Lancrenon, 1996; Pinto et al., 2008; Stein et al., 2007, 2008; Wu et al., 2007). Although the factors identified in these studies overlap with those found in category-based analyses, there are some key differences, i.e. the identification of 5 rather than 4 factors: 1) symmetry and repeating, ordering and counting; 2) aggressive, sexual and religious obsessions; 3) contamination and cleaning; 4) aggressive obsessions and checking, and 5) somatic obsessions.(Bloch et al., 2008a).

Three studies have performed item-level confirmatory factor analysis (CFA) of OC symptoms (Stewart et al., 2008; Summerfeldt et al., 1999; Wu et al., 2007). These studies investigated the fit of the original three factor model described by Baer (1994) containing: 1) hoarding, symmetry behavior, and counting; 2) contamination, cleaning, somatic obsessions, checking; and 3) aggressive, sexual and religious obsessions; the "classical" four factor model described by Leckman et al (1997); the five factor model found by Mataix-Cols et al. (1999), and a two factor model (obsessions versus compulsions) (Stewart et al. 2008; Summerfeldt et al. 1999). Overall, the four factor model showed the best fit, although it was not entirely ideal.

Although OCD is to some extent heritable, it is etiologically complex (Pauls, 2008). OC symptom dimensions may be more heritable than OCD per se (Hasler et al., 2007; Pauls and Alsobrook, 1999). Although some studies examining the heritability of OC symptom dimensions have been published, this area has not yet been investigated comprehensively, and results have been inconsistent. An early family study suggested that symmetry and ordering symptoms had a significant genetic component (Alsobrook et al., 1999), and two subsequent studies found significant intra-class correlations in independent sib pairs for symmetry/ordering and hoarding (Cullen et al., 2007) and for contamination/cleaning symptoms and hoarding respectively (Chacon et al. 2007). In the OCD Collaborative Genetics Study, significant sib-sib associations were found for four of the five factors, with hoarding and taboo thoughts being the most robustly familial (Hasler et al. 2007; Pinto et al., 2008). At the same time, heritability for OC

symptoms in general may be greater than for any particular symptom dimension. In multigenerational families with OCD and hoarding, OC symptoms showed higher heritability than hoarding (Mathews et al., 2007b). Further, in a population-based twin study all symptom dimensions shared variation with one latent common factor (i.e., OC behavior), and variation within this common factor was explained by both genes (36%) and environmental factors (64%). Only the contamination dimension was influenced by specific genes and seemed relatively independent (van Grootheest et al., 2008). A number of genetic studies have found links between candidate genes or chromosomal regions and symptom dimensions, particularly hoarding, although again data are not always consistent (Alonso et al., 2008b; Lochner et al., 2005b; Samuels et al., 2007; Zhang et al., 2002). Taken together, these various studies support the potential value of assessing symptom dimensions, but, in addition to this, call for elucidation of the findings on the OCD phenotype as a whole.

In summary, although there is overlap between findings, the factor analyses of OC symptoms published to date have not yet fully converged, due to insufficient sample size and differences in analytic approaches. In addition, the heritability of the various OC symptom dimensions has not been fully examined, thus their usefulness as alternative phenotypes for genetic studies is still unclear. The aims of this study were to 1) identify homogeneous symptom dimensions through item-level factor analysis in a large heterogeneous sample of OCD patients, 2) to conduct heritability analyses on the resulting factors in a subset of OCD families, and 3) to examine potential associations between the symptom dimensions and selected clinical characteristics. This study has three primary design advantages over previously published studies in this area, including: 1) large sample size, allowing for more precise and stable factor solutions; 2) improved statistical methodology, i.e. the use of model fitting procedures in Mplus using full information maximum likelihood model fitting, which allows for improved handling of dichotomous data and missing values; and 3) heritability analyses in large multigenerational families with multiple OCD-affected individuals, improving the precision of heritability estimates.

Table 1. Exploratory item-level factor analytic studies in OCD.

| Study | Subjects (n) | Number of factors | Contamination/ Cleaning | Aggressive/ Harm | Superstitions | Somatic | Hoarding | Sexual/ Religious | Symmetry |
|---|---|---|---|---|---|---|---|---|---|
| Hantouche et al., 1996 | 615 | 17 | X | X | X | X | X | X | X |
| Girishchandra et al., 2001 | 202 | 5 | X | X (including checking) | X | | X (including symmetry) | X | |
| Feinstein et al., 2003 | 160 | 4 | X | X (including checking) | | | X (including symmetry) | X | |
| Denys et al., 2004b | 335 | 5 | X | X (including checking) | | X | | X (including aggressive obsessions) | X (including hoarding) |
| Wu et al., 2008 | 149 general psychiatric patients+ 805 controls | 3 | X | X (including sexual/ religious obsessions) | | | | | X (including checking/ arranging/ counting/ hoarding |
| Stein et al., 2007 | 434 | 5 | X | X (including sexual/ religious obsessions) | | X | X (including symmetry) | X (including aggressive obsessions) | X (including hoarding) |
| Pinto et al., 2008 | 485 | 5 | X | X (including checking) | | | X | X (including aggressive obsessions) | X (including hoarding |
| Stein et al., 2008 | 466 | 5 | X | X (including checking) | | X | | X | X (including hoarding) |

**Methods**

This project encompasses a joint venture between the Department of Psychiatry of the University of California in San Francisco, the Department of Psychiatry of the Massachusetts General Hospital and McLean Hospital, GGZ Buitenamstel Amsterdam, the Department of Psychiatry of the University Medical Center Groningen, the MRC Research Unit on Anxiety Disorders and the MRC/US Centre for Molecular and Cellular Biology, University of Stellenbosch in South Africa, and the Departments of Psychiatry, Neurology, and Laboratory Medicine at the University of Minnesota.

Data from the YBOCS-CL were pooled for analyses from 1224 study participants collected at five sites: San Francisco (n = 124), Boston (n = 329), Amsterdam (n = 229), Stellenbosch (n = 393), and Groningen (n = 149). All subjects met criteria for a lifetime diagnosis of OCD according to DSM-IV criteria (American Psychiatric Association, 1994).

The San Francisco sample was recruited for genetic and phenomenological studies and has been described previously (Chavira et al., 2008). Subjects from Boston were patients on their first admission to the Massachusetts General Hospital/McLean OCD Institute. A subpopulation of these subjects participated in a study of the effectiveness of intensive residential treatment for severe, refractory OCD (Stewart et al., 2005). Subjects from Amsterdam were recruited from an anxiety outpatient clinic. Subjects from Stellenbosch were recruited by physician referral, media advertisements, the Mental Health Information Centre (MHIC) and the OCD Association of South Africa (OCDSA), as described previously (Hemmings et al., 2008). The Groningen subjects were recruited for genetic and treatment studies of OCD by physician referral, media advertisements and the Dutch patient association for anxiety disorders. The sample for the heritability analyses included 52 OCD families (258 individuals in total) collected by the San Francisco and Minnesota sites. Only one proband from each family was included in the factor analyses. The majority of the study population (> 90%) was of Caucasian descent.

All diagnoses were established according to DSM-IV criteria (American Psychiatric Association, 1994) using the Structured Clinical Interview for Axis I disorders (SCID-I/P) (First et al., 1998), the Mini International Neuropsychiatric Interview (MINI) version 5.0.0 (Sheehan et al., 1998), or expert clinician diagnosis (Stewart et al., 2005). The study was approved by the Medical Ethical Review boards of all participating centers. All subjects (in addition to the parents of minors) gave written informed consent for participation in the study.

*Data collection*

The YBOCS-CL was used to assess OC symptoms (Goodman et al., 1989a, 1989b). Items were coded 0 when the patient never had the symptom and 1 when the symptom was reported in past and/or present. The 74 items of the original published version (Goodman et al., 1989b) were used in the analyses.

The Yale-Brown Obsessive-Compulsive Severity Scale (YBOCS-SS) (Goodman et al., 1989a) was used to assess worst ever (San Francisco) or present (Groningen, Amsterdam, Stellenbosch and Boston) severity of OC symptoms. In addition, age of onset of OC symptoms was determined. Family history of OC symptoms (i.e., the presence of OC symptoms in at least one family member) was recorded for the Boston and Amsterdam sites.

*Analysis Methods*

The distribution of all 74 YBOCS-CL items was examined. Ten of the items labeled "other" were excluded from analysis due to their heterogeneity (e.g., "other contamination obsessions"). The YBOCS-CL has two symmetry items: "symmetry obsession accompanied by magical thinking" and "symmetry obsession not accompanied by magical thinking". The YBOCS-CL version used in San Francisco and in a subset of 100 patients from Amsterdam contained several symmetry items but did not make a distinction on presence or absence of magical thinking. Therefore, one symmetry item was created for all patients, representing the presence or absence of any symmetry obsession. This ultimately resulted in the inclusion of 63 YBOCS-CL items in the exploratory factor analysis.

Exploratory and confirmatory factor analyses

Exploratory principal component factor analyses with varimax rotation were conducted on the 63 YBOCS-CL items using SAS (v9.1.3) with tetrachoric correlation coefficient estimates used for the item-level correlation matrix (via the %polychor macro). All available data were used in the analyses. Varimax rotation was used for ease of interpretation and to produce the clearest distinctions between the resulting factors. The scree plot was examined, and only factors with an eigenvalue >1 were retained.

Confirmatory factor analyses for models comprising 4-8 factors were then performed using Mplus (v4.2) to determine the model with the most parsimonious fit. In these analyses, the factor analysis estimation was based upon weighted least-squares estimates using a diagonal weight matrix. Items were assigned to a factor if they had a loading $\geq 0.4$ on that factor. Items that cross-loaded (i.e., had a loading of $> 0.3$ on three or more factors), items that did not load $\geq 0.4$ on any factor, and items that were

unstable across models (i.e., loaded on very different factors from model to model) were omitted from the confirmatory analyses.

To establish the number of factors and factor constitution for the best-fit model, the following fit indices were used: the chi-square fit-statistic, the comparative fit index (CFI), the Tucker-Lewis Index (TLI), the Root Mean Square Error of Approximation (RMSEA) and the Standardized Root Mean Square Residual (SRMR). Values of the CFI and of TLI approaching 0.95, values approaching 0.08 of the SRMR, and values of the RMSEA < 0.05 are generally indicative of a good fit (Browne and Cudek, 1993; Hu and Bentler, 1999).

In addition, a principal component analysis using the category-based approach and a promax rotation was performed for purposes of comparison (with the item-level FA). For this analysis, missing items in the YBOCS-CL severity scale were imputed using Solas™ 3.2 (statistical solutions, ltd; Cork, Ireland) using predictive model-based imputation. Missing variables were imputed five times, yielding five complete datasets, as recommended by Schafer (Schafer, 1999).

Creation of mean sum scores

Since the factor scores generated by a factor analysis are specific to their dataset of origin and are not readily generalizable to other datasets, mean scale scores for the resulting factors were calculated in the final model. This was conducted by dividing the number of items endorsed in the factor by the total number of items in the factor for each subject. The total number of items endorsed by each individual (total sum score), was also calculated. These scores were then used in the heritability analyses and in correlation analyses with demographic and other clinical characteristics.

Heritability analyses

Heritability estimates and the corresponding significance levels for the mean scale scores were calculated for each factor and for two global measures, the YBOCS severity score and the total sum score, using the Sequential Oligogenic Linkage Analysis Routine (SOLAR) statistical package, version 4.0.7 (Almasy and Blangero, 1998). SOLAR employs a variance components approach that uses information from all available family members across generations and does not assume an inheritance model. The resultant heritability statistic ($h^2r$) is based on a maximum-likelihood-based variance decomposition approach providing an estimate and a confidence interval. Proband status was controlled for in all analyses, and age at interview and gender were included as covariates. Since neither factor scale scores nor the total sum score were normally distributed, these variables were transformed using inverse normal

transformations prior to calculating heritabilities. Pair-wise genetic and environmental correlations with corresponding standard errors and significance values were calculated between the symptom factors and YBOCS severity score to explore their etiological relationships.

Correlation of factor scores with other clinical phenotypes

The relation between mean scores for the factors and gender, age of OC symptom onset and age at assessment were estimated using multiple linear regression analyses. Age of onset, sex and age at assessment were modeled in multiple regression analyses with mean scale scores as the dependent variable.

**Results**

Demographics and clinical data are summarized in table 2. The total sample consisted of 1224 subjects. 50.5% (n=618) of the subjects were female. Mean age at assessment (± SD) was 33.8 ± 12.6 years. Mean age of symptom onset (± SD) was 16.0 ± 9.2 years. Mean total YBOCS severity (± SD) was 22.6 ± 8.6. Data on family history were available for 431 patients, of whom 157 (36.4%) had a positive family history for OC symptoms.

The U.S. samples included more males than the Dutch or South-African sample ($p < 0.001$), had a lower age of onset of OC symptoms ($p < 0.001$), and higher YBOCS total ($p < 0.001$), obsession ($p < 0.001$) and compulsion ($p < 0.001$) subscale scores and less often had a positive family history for OC symptoms ($p < 0.001$). Endorsement rates of individual items also differed between sites, being higher in U.S. versus Dutch/South African subjects for all except 9 items, mostly encompassing miscellaneous items.

Table 2. Subject demographics and clinical characteristics by recruitment site.*

| | n | Sex (females) (%) | Age at assessment (y) | Age of OC symptom onset (y) | YBOCS severity score | YBOCS obsession score | YBOCS compulsion score | Positive family history |
|---|---|---|---|---|---|---|---|---|
| Boston | 329 | (41.5%) | 32.4±11.3 | 15.4±8.2 | 27.9±6.3 | 14.2±3.5 | 13.7±3.7 | 29.1% |
| S. Francisco | 124 | (46.8%) | 31.9±13.8 | 8.8±4.3 | 27.1±7.3 | 13.7±3.8 | 13.4±4. | NA |
| Groningen | 149 | (63.8%) | 38.7±11.9 | 19.0±10.6 | 17.4±7.9 | 8.2±4.4 | 9.1±4.7 | NA |
| South Africa | 393 | (49.6%) | 32.2±13.7 | 16.7±9.9 | 19.0±8.2 | NA | NA | NA |
| Amsterdam | 229 | (56.3%) | 36.0±10.9 | 16.7±8.3 | 23.3±7.9 | 11.4±4.2 | 11.9±4.7 | 48.2% |
| Total cohort | 1224 | (50.5%) | 33.8±12.6 | 16.0±9.2 | 22.6±8.6 | 12.1±4.5 | 12.2±4.6 | 36.4% |

* NA= Not available

Item-level factor analyses

In the initial exploratory factor analysis, there were 11 eigenvalues ≥1, accounting for 73.3% of the total variance, and 5 eigenvalues ≥2. The first eigenvalue (17.45) accounted for 32% of the variance; the second (5.2) accounted for 10% of the additional variance. Examination of the scree plot and eigenvalues from the initial exploratory factor analysis suggested that the most likely best-fit models contained between 4 and 6 factors. Because the scree plot dropped abruptly after the first factor, confirmatory factor analyses were conducted for 1, 4, 5, and 6 factors. Of the 63 items that were included in the exploratory analyses, 10 items were omitted from the confirmatory factor analyses because they cross-loaded (i.e., had a loading of > 0.3 on three or more factors), did not load ≥ 0.4 on any factor, or were unstable across models. These included the items "nonsense sounds", "bothered by certain sounds", "need to tell, ask or confess", "need to touch or rub", "rituals involving blinking or staring", "trichotillomania", "other self-damaging behavior", "excessive concern with illness/disease", "excessive concern with bodily part or appearance", and "checking tied to somatic obsessions". This resulted in the inclusion of 53 items in the confirmatory factor analyses.

Fit indices for the 1, 4, 5 and 6 factor models are summarized in table 3. A five factor model showed the best fit. The five factors obtained included: 1) taboo (sexual, aggressive and religious symptoms), 2) contamination/cleaning (contamination obsessions and cleaning compulsions), 3) doubts (obsessions related to fears of having caused harm to self or others, and checking compulsions related to these fears), 4) rituals/superstition (superstitious obsessions and compulsions such as lucky numbers or colors, rituals such as ritualized eating behaviors, and mental rituals) and 5) symmetry/hoarding (hoarding obsessions and compulsions, symmetry, ordering and arranging compulsions, and symptoms related to fear of losing things or making an error). Cronbach's alpha coefficients ranged between 0.73 and 0.87, indicating good internal consistency of the factors and scale intercorrelations ranged between 0.36 and 0.64 (all statistically significant at $p < 0.0001$). Item loadings for the five factor model are displayed in table 4.

Table 3. Confirmatory factor analyses fit indices for the 1, 4, 5 and 6 factor solutions.

| **Number of factors** | **1** | **4** | **5** | **6** |
|---|---|---|---|---|
| CFI | 0.384 | 0.724 | 0.746 | 0.742 |
| TLI | 0.768 | 0.902 | 0.911 | 0.910 |
| RMSEA | 0.114 | 0.074 | 0.071 | 0.071 |
| WRMR | 3.424 | 2.312 | 2.203 | 0.2000 |
| $\chi^2$ (df) | 1168.21 | 180.546 (4) | 8.959 (4) | -- |
| p-value | <0.0001 | <0.0001 | 0.0624 | -- |

CFI: comparative fit index; TLI: Tucker-Lewis Index; RMSEA; Root Mean Square Error of Approximation; WRMR: Weighted Root Mean square Residual.

### Categorical Factor Analysis

Categorical factor analysis using 13 of the 15 predefined symptom categories, and excluding the miscellaneous items, yielded 4 factors explaining 65.5% of the variance (data not shown): Factor 1: symmetry obsessions, ordering and arranging, counting and repeating compulsions; Factor 2: aggressive, sexual and religious obsessions and checking compulsions; Factor 3: contamination obsessions and cleaning compulsions; and Factor 4: hoarding obsession and compulsions.

Table 4. Factor loadings, eigenvalues and variance explained for the best fit five factor model*.

| | **Factor 1** Taboo | **Factor 2** Contamination/ cleaning | **Factor 3** Doubts | **Factor 4** Rituals/ superstition | **Factor 5** Symmetry/ hoarding |
|---|---|---|---|---|---|
| Forbidden/perverse thoughts, images, impulses | **0.88** | 0.10 | 0.02 | 0.10 | 0.00 |
| Content involves children on incest | **0.84** | 0.15 | 0.03 | 0.04 | -0.09 |
| Sexual behavior towards others | **0.82** | 0.14 | 0.07 | 0.22 | 0.12 |
| Obsessions involving homosexuality | **0.81** | 0.17 | 0.00 | 0.21 | -0.10 |
| Violent or horrific images | **0.70** | 0.09 | 0.17 | 0.17 | 0.11 |
| Fear will act on unwanted impulses | **0.70** | 0.00 | 0.38 | 0.05 | 0.14 |
| Fear of blurting obscenities/insults | **0.66** | 0.08 | 0.31 | 0.00 | 0.21 |
| Fear will steal things | **0.62** | 0.27 | 0.27 | 0.09 | 0.38 |
| Fear of doing something embarrassing | **0.60** | 0.12 | 0.25 | 0.08 | 0.38 |
| Fear might harm self | **0.58** | 0.09 | 0.20 | 0.26 | 0.10 |
| Concern with sacrilege/blasphemy | **0.56** | 0.20 | 0.17 | 0.26 | 0.01 |
| Intrusive (nonviolent) images | **0.52** | 0.14 | 0.01 | **0.41** | 0.12 |
| Concern with right/wrong, morality | **0.48** | 0.14 | 0.30 | 0.28 | 0.10 |
| Fear of saying certain things | **0.41** | 0.13 | 0.31 | 0.39 | 0.27 |
| Concern with dirt or germs | 0.10 | **0.92** | 0.13 | 0.03 | 0.06 |
| Excessive/ ritualized hand washing | -0.04 | **0.88** | 0.15 | 0.16 | 0.01 |
| Other measures to prevent/remove contaminants | 0.12 | **0.81** | 0.10 | 0.19 | 0.03 |
| Concerns with bodily secretions | 0.27 | **0.78** | 0.16 | 0.08 | 0.01 |
| Cleaning household items/ objects | -0.04 | **0.74** | 0.06 | 0.15 | 0.26 |
| Excessive showering, grooming | -0.03 | **0.73** | 0.02 | 0.25 | 0.24 |
| Excessive concern with animals | 0.23 | **0.73** | 0.10 | 0.10 | 0.17 |
| Bothered by sticky substances | 0.13 | **0.72** | 0.02 | 0.19 | 0.19 |
| Concern with household cleaners | 0.20 | **0.70** | 0.21 | 0.14 | 0.17 |
| Concern with environmental contaminants | 0.26 | **0.66** | 0.24 | 0.12 | 0.00 |
| Concerned will get ill because of contaminant | 0.26 | **0.62** | 0.33 | 0.12 | -0.02 |
| Concern about contaminant no other than how it might feel | 0.02 | **0.57** | -0.20 | -0.07 | 0.17 |

*Numbers depicted in bold indicate items with factor loadings > 0.4 and that were included in the corresponding factor in the calculation of the mean score per items for this factor

| | **Factor 1** Taboo | **Factor 2** Contamination/ cleaning | **Factor 3** Doubts | **Factor 4** Rituals/ superstition | **Factor 5** Symmetry/ hoarding |
|---|---|---|---|---|---|
| Checking that did not harm others | 0.32 | 0.10 | **0.81** | 0.12 | -0.02 |
| Fear will be responsible for something terrible happening | 0.19 | 0.10 | **0.76** | 0.15 | 0.08 |
| Fear might harm others | 0.37 | 0.20 | **0.73** | 0.05 | 0.09 |
| Checking that nothing terrible did/will happen | 0.25 | 0.13 | **0.72** | 0.27 | 0.06 |
| Checking locks, stoves, appliances | -0.19 | 0.09 | **0.63** | 0.08 | 0.30 |
| Checking that did not make mistake | 0.06 | 0.16 | **0.60** | 0.15 | **0.44** |
| Fear might harm others because not careful enough | **0.59** | -0.02 | **0.59** | 0.00 | 0.03 |
| Checking did not harm self | 0.37 | 0.27 | **0.53** | 0.25 | 0.15 |
| Concerned will make others ill | 0.29 | **0.49** | **0.51** | 0.10 | -0.06 |
| Superstitious behaviors | 0.20 | 0.20 | 0.14 | **0.76** | 0.08 |
| Lucky/unlucky numbers | 0.18 | 0.11 | 0.07 | **0.73** | 0.16 |
| Superstitious fears | 0.21 | 0.17 | 0.17 | **0.73** | -0.02 |
| Need to repeat routine activities | 0.00 | 0.05 | 0.11 | **0.69** | 0.28 |
| Colors with special significance | 0.19 | 0.23 | 0.11 | **0.60** | 0.11 |
| Counting compulsions | 0.05 | 0.08 | 0.11 | **0.52** | **0.42** |
| Ritualized eating behaviors | 0.21 | 0.23 | -0.02 | **0.48** | 0.33 |
| Mental rituals | 0.34 | 0.05 | 0.13 | **0.45** | 0.20 |
| Measures (not checking) to prevent harm or terrible consequences | 0.32 | 0.13 | 0.39 | **0.43** | -0.04 |
| Ordering/arranging compulsions | 0.00 | 0.18 | -0.03 | 0.27 | **0.71** |
| Hoarding obsessions | 0.25 | 0.17 | 0.04 | 0.12 | **0.63** |
| Symmetry obsessions | -0.03 | 0.18 | -0.07 | 0.22 | **0.62** |
| Hoarding compulsions | 0.20 | 0.14 | 0.06 | 0.16 | **0.62** |
| Excessive listmaking | -0.09 | -0.06 | 0.11 | -0.12 | **0.59** |
| Fear of losing things | 0.18 | 0.12 | 0.37 | 0.17 | **0.58** |
| Re-reading, re-writing | 0.08 | 0.13 | 0.19 | **0.45** | **0.49** |
| Need to know, remember | 0.34 | 0.10 | 0.15 | 0.30 | **0.46** |
| Fear of not saying the right thing | 0.27 | 0.18 | 0.26 | 0.23 | **0.42** |
| Cronbach's alpha | 0.85 | 0.87 | 0.81 | 0.74 | 0.73 |
| Eigenvalue | 17.5 | 5.2 | 3.9 | 2.9 | 2.1 |
| Variance explained (%) | 0.32 | 0.10 | 0.07 | 0.05 | 0.04 |
| Cumulative variance explained (%) | 0.32 | 0.42 | 0.49 | 0.55 | 0.58 |

*Numbers depicted in bold indicate items with factor loadings > 0.4 and that were included in the corresponding factor in the calculation of the mean score per items for this factor

Heritability analyses:

Heritability analyses were conducted in 52 OCD families (mean number of individuals per family = 5, range = 2-40) (table 5). YBOCS severity and YBOCS total item number were both heritable, as were all symptom factors except for the rituals/superstition factor, with heritability estimates ranging between 0.28 and 0.52. All symptom factors showed shared environmental influences with YBOCS severity, with estimates (RhoE) ranging between 0.36 and 0.63 (table 6). The symmetry/ hoarding factor and the contamination/cleaning factor also shared genetic influences with YBOCS severity, with RhoGs of 0.78 (p=0.0005) and 0.48 (p=0.04), respectively.

Table 5. Heritabilities for mean factor sum scores of the YBOCS-CL.

| | | | | | covariate p-values | |
|---|---|---|---|---|---|---|
| **Phenotype** | **$h^2r$** | **SE** | **p-value** | **kurtosis** | **age** | **sex** |
| Factor 1 (Taboo) factor scale score | 0.28 | 0.12 | 0.005 | -0.65 | 0.30 | 0.15 |
| Factor 2 (Contamination/cleaning) factor scale score | 0.35 | 0.13 | 0.002 | -0.55 | 0.41 | 0.05 |
| Factor 3 (Doubts) factor scale score | 0.40 | 0.14 | 0.0004 | -0.68 | 0.63 | 0.14 |
| Factor 4 (Rituals/superstition) factor scale score | 0.06 | 0.09 | 0.23 | -0.43 | 0.21 | 0.0008 |
| Factor 5 (Symmetry/hoarding) factor scale score | 0.37 | 0.14 | 0.002 | -0.74 | 0.49 | 0.03 |
| YBOCS total sum score | 0.39 | 0.15 | 0.004 | -0.43 | 0.73 | 0.03 |
| YBOCS total severity score | 0.52 | 0.13 | 0.00005 | -0.98 | 0.35 | 0.001 |

H2r = heritability estimate; SE= Standard Error; Total sum score represents the average of all YBOCS-CL items included in the factor analysis

Table 6. Bivariate genetic analyses with 5 factor solution and YBOCS total severity scores.

| **Phenotype** | **N** | **RhoG** | **SE** | **p-value** | **RhoE** | **SE** | **p-value** |
|---|---|---|---|---|---|---|---|
| Factor 1 (Taboo) factor scale score | 257 | 0.41 | 0.24 | 0.11 | 0.36 | 0.15 | 0.03 |
| Factor 2 (Contamination/cleaning) factor scale score | 258 | 0.48 | 0.19 | 0.04 | 0.41 | 0.14 | 0.01 |
| Factor 3 (Doubts) factor scale score | 258 | 0.39 | 0.19 | 0.09 | 0.44 | 0.14 | 0.009 |
| Factor 4 (Rituals/superstition) factor scale score | 258 | 0.15 | 0.41 | 0.72 | 0.63 | 0.11 | $3.4 \times e^{-6}$ |
| Factor 5 (Symmetry/hoarding) factor scale score | 258 | 0.78 | 0.10 | 0.0005 | 0.43 | 0.13 | 0.02 |
| YBOCS total sum score | 258 | 0.72 | 0.13 | 0.002 | 0.57 | 0.12 | 0.001 |

RhoG = shared genetic variance; RhoE =shared environmental variance; SE = Standard Error.

Correlations of factor scores with clinical characteristics

For all factors, higher mean sum scores were associated with earlier age of onset (table 7). Mean sum scores for the taboo factor were significantly higher in males than in females, and the taboo and symmetry/hoarding factors were negatively associated with age at assessment.

Table 7. Regression analyses of mean item scores with sex, age at assessment, and age of onset of obsessive-compulsive symptoms as predictors.*

| **Multiple regression model** (n = 1039) | $R^2$ | **sex** | **Age at assessment** | **Age of onset** |
|---|---|---|---|---|
| Factor 1 (Taboo) factor scale score | 0.066 | **p < 0.001** | **p = 0.003** | **p < 0.001** |
| Factor 2 (Contamination/cleaning) factor scale score | 0.023 | p = 0.747 | p = 0.058 | **p < 0.001** |
| Factor 3 (Doubts) factor scale score | 0.016 | p = 0.591 | p = 0.840 | **p < 0.001** |
| Factor 4 (Rituals/superstitions) factor scale score | 0.058 | p = 0.065 | p = 0.542 | **p < 0.001** |
| Factor 5 (Symmetry/hoarding) factor scale score | 0.039 | p = 0.719 | **p = 0.003** | **p < 0.001** |

* Gender: 0 = male, 1 = female; Family history: 0 = negative, 1 = positive

**Discussion**

Consistent with previous item-level factor-analytic studies, this study, which has the largest sample size published to date, identified five symptom factors, including 1) taboo (religious, sexual, and some aggressive obsessions), 2) contamination and cleaning symptoms, 3) doubts (fears of harming self or others, doubting and checking symptoms), 4) superstitions and rituals, and 5) symmetry and hoarding (hoarding, perfectionism, and symmetry behaviors). This symptom dimension model shows substantial overlap with the factor structure identified in previous item-level analyses, with two key differences (Denys et al., 2004b; Feinstein et al., 2003; Girishchandra and Khanna, 2001; Hantouche and Lancrenon, 1996; Pinto et al., 2008; Stein et al., 2007; Stein et al., 2008; Wu et al., 2007). First, in contrast to most previous studies, but consistent with a recent study by Cullen et al. (2007) we found that somatic items on the YBOCS-CL did not load consistently on any of the factors: a better fit and more stable solutions were obtained when these items were omitted from the analyses. This finding suggests that some somatic items, although frequently clinically co-occurring with OCD, do not represent core OC symptoms, and might contribute to the observed phenotypic and genotypic heterogeneity. Instead, the somatic items may be related to hypochondriasis, which is frequently comorbid with OCD (Bienvenu et al., 2000). Recent phenomenological and neuroimaging studies have shown that, although hypochondriasis and OCD might share some phenotypic and brain function characteristics, they are also clearly distinct (Greeven et al., 2006; van den Heuvel et al., 2005). Second, the rituals/superstition factor found in the current study is new, and contains predominantly obsessions and compulsions that are in the miscellaneous section of the YBOCS and were not included in most previous factor analytic studies. However, considering the fact that this symptom dimension was not heritable in our families, possibly due to its heterogeneity, this symptom dimension is unlikely to be informative in genetic studies. Although useful for clinical (treatment) studies, one might consider omitting this symptom dimension from future genetic studies.

The stability of the remaining symptoms within factors across various models, the high internal consistency of the factors, and the high heritability estimates of the factors provide additional evidence that OCD may be dissected into consistent and more homogeneous symptom dimensions. Of note, the oatic factor analysis yielded similar results to those previously reported in other samples (rather than being similar to the item-level analysis on this sample). This suggests that, although the category-based model is stable across studies, it is limited as it does not take advantage of all available data. Item-level factor analysis seems to yield more homogeneous results than category-based factor analysis. Previous heritability studies that used category-based

analyses predominantly used sib-sib correlations, yielding mixed results, especially with respect to heritability estimates of the symmetry/ordering factor (Chacon et al., 2007; Cullen et al., 2007; Hasler et al., 2007). Interestingly, consistent with the first categorical FA's in OCD (Baer, 1994), hoarding and symmetry behaviors constituted one heritable factor in this study. Moreover, the symmetry/hoarding factor had the highest genetic correlation with a latent or underlying OC susceptibility (as measured by YBOCS severity). This may be an artifact of the available family data, which contained a substantial number of hoarding families, or it may alternatively reflect a true relationship between these constructs. In either event, this finding, along with all of the heritability results, requires replication in an independent sample.

Notably, although not the best fit for the data, a one-factor model also emerged as a possible solution according to the eigen structure as evidenced by the scree plot. When combined with the heritability results, these results suggest the presence of one heritable underlying susceptibility to OC symptoms in general (as measured by the total item number and YBOCS severity). These findings are in line with a recent population-based twin study of OC symptom dimensions, and with a family study of OC symptoms in extended families, both of which found evidence of one heritable underlying latent susceptibility to OC symptoms along with specific genetic contributions to OC symptom dimensions (Mathews et al., 2008; van Grootheest et al., 2008).

The negative associations between mean factor item scores and YBOCS severity and age of onset are in concordance with previous research reporting a higher number of obsessions and compulsions and higher symptom severity in subjects with early onset compared to subjects with late onset OCD (Millet et al., 2004). Finally, corroborating the literature (Torresan et al., 2009), men showed higher mean scores on the "Taboo" factor (aggressive and sexual obsessions) than women. Apparently, this predominance of aggressive and sexual obsessions in men holds across cultures (Jaisoorya et al., 2009). The overrepresentation of the "rituals and superstition" factor in men compared to women has been reported in one study (Jaisoorya et al., 2009), and adds to the reported gender differences with respect to phenotypic expression of OCD.

The main methodological strengths of this study are the large sample size, and the use of improved statistical methodology. The heterogeneity of the sample is the principal limitation of this study, and also, paradoxically its principal strength. This sample heterogeneity may reflect differences in data collection and ascertainment strategies. Although such heterogeneity may theoretically contribute to the instability of resulting models, we found that the ultimate best-fit model was remarkably stable and most likely a result of the large sample size. In fact, the sample heterogeneity increases

generalizability of the findings, which is an additional strength. The number of probands for whom family data were available was relatively small, which decreases the power and interpretability of the heritability analyses.

In summary, in the face of an emerging need to refine the OCD phenotype for genetic and other etiological studies, our results support the ongoing usefulness of the OCD phenotype as a whole and also of specific symptom subgroups (Leckman and Bloch 2008). The new heritability results on OCD with its symptom dimensions raise the possibility that the genes and gene networks underlying common susceptibility to OCD differ from the genes and gene networks underlying the different symptom dimensions.

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Chapter 3

# Latent Class Analysis of YBOCS Symptoms in Obsessive Compulsive Disorder

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*Submitted.*

**Abstract**

***Objective:*** *Obsessive-compulsive disorder (OCD) is phenomenologically heterogeneous, and findings of underlying structure classification based on symptom grouping have been ambiguous to date. Variable-centered approaches, primarily factor analysis, have been used to identify homogeneous groups of symptoms, but person-centered latent methods have seen little use. This study was designed to uncover sets of homogeneous groupings of 1611 individuals with OCD, based on symptoms.*

***Method:*** *Latent class analysis (LCA) models using 61 obsessive-compulsive symptoms (OCS) collected from the Yale-Brown Obsessive-Compulsive Scale were fit. Relationships between latent class membership and treatment response, gender, symptom severity and comorbid tics were tested for relationship to class membership.*

***Results:*** *LCA models of best fit yielded either three or five classes. Classes in the three-class solution differed only in frequency of endorsement, indicating individuals vary only in level of severity. The five-class solution was similar to the three-class, with the addition of a minimal endorsement class and, interestingly, a class with predominantly contamination/cleaning symptoms. Classes with higher symptom endorsement were associated with earlier age of onset, being male, and comorbid tics.*

***Conclusions****: These results provide support for the validity of OCD as a single diagnostic entity, and are in line with genetic epidemiology studies suggesting an underlying latent susceptibility to OCD.*

## Introduction

Obsessive-compulsive disorder (OCD) is a common neuropsychiatric disorder, affecting 2% of adults and between 1% and 2% of children (Bebbington, 1998; Horwath and Weissman, 2000; Ruscio et al., 2008). Although the DSM-IV-TR definition is straightforward, OCD is phenomenologically heterogeneous and etiologically complex (Alsobrook et al., 1999; American Psychiatric Association, 2000; Cavallini et al., 1999; do Rosario-Campos et al., 2005; Hanna et al., 2005b; Nestadt et al., 2000a). OCD-affected individuals exhibit a wide variety of symptoms and a range of comorbid neuropsychiatric conditions.

In attempting to better understand OCD, investigators have used data reduction methods, most commonly factor analysis, to identify subgroups of symptoms (as defined by the Yale Brown Obsessive Compulsive Scale (YBOCS) symptom checklist) that may be amenable to etiological and treatment studies (Alsobrook et al., 1999; Baer, 1994; Cavallini et al., 2002; Delorme et al., 2006a; Denys et al., 2004a, 2004b; Feinstein et al., 2003; Hasler et al., 2005, 2006; Lochner et al., 2005a; Lochner and Stein, 2006; Mataix-Cols et al., 1999; Matsunaga et al., 2008; McKay et al., 1995; Nestadt et al., 2003; Pinto et al., 2007; Stein et al., 2007; Summerfeldt et al., 1999; Thomsen and Jensen, 1991). However, while factor analysis seeks the underlying structure in variables, other approaches, such as latent class analysis (LCA), can be used to find latent homogeneous groups of individuals and provide an additional dimension of analysis, potentially refining the phenotype. While LCA has been used on symptom data from other neuropsychiatric disorders, most notably attention deficit-hyperactivity disorder (Hudziak et al., 1998; Lubke et al., 2007; Neuman et al., 1999, 2001; Rasmussen et al., 2002a, 2002b, 2004; Rohde et al., 2001), studies examining the latent class structure of obsessive-compulsive symptoms are non-existent.

In a pioneering application of LCA to OCD, Thomsen and Jensen used four variables in an LCA (i.e. neurological signs, EEG abnormalities, attention deficit and developmental disorder) to examine birth complications and neurological abnormalities in individuals with OCD and controls (Thomsen and Jensen, 1991). With only four measures they were limited to at most a two-class model, which they identified as an "organic class" and a "non-organic class"; individuals with OCD primarily fell into the "non-organic class", suggesting that OCD is not likely to be the result of organic brain disease. The two other studies that used this approach in OCD examined latent classes based on patterns of comorbidity but not symptoms (Nestadt et al., 2003; Nestadt et al., 2008; Thomsen and Jensen, 1991).

Given this lack of research on the grouping of individuals by symptomatology, the primary aim of this study was to examine latent groups based on obsessive-compulsive (OC) symptoms using LCA in a large heterogeneous sample, and to examine the relevance of the derived latent classes by assessing their relationships to clinically relevant variables such as gender, symptom severity, presence or absence of comorbid tics, family history of OCD symptoms, and treatment response. We hypothesized that the LCA would parallel the observed clinical heterogeneity, that is, that the latent classes would be characterized by groups of items which were highly endorsed for one class and not for the others, for example, a group endorsing only the hoarding-related items and one endorsing mainly the items related to contamination. Further, we hypothesized that the latent classes would have specific clinical and/or demographic profiles (e.g., some identified latent classes would have an earlier age of onset of symptoms or be more prevalent in women).

## Method

### *Participants*

The study sample used in these analyses has been described in detail previously (Chavira et al., 2008; Denys et al., 2004b; Katerberg et al., 2009b; Mathews et al., 2007b; Stewart et al., 2005). Briefly, subjects who met criteria for a lifetime diagnosis of OCD according to DSM-IV criteria (n=1611) were recruited from six sites, the Department of Psychiatry of the University of California, San Francisco (n=124), the Department of Psychiatry of the McLean/Massachusetts General Hospital (n=329), the Department of Psychiatry of the VU Medical Center and the Outpatient Academic Clinic for Anxiety Disorders, GGZ Buitenamstel in Amsterdam (n=229), the Department of Psychiatry of the Amsterdam Medical Center (n=387), the MRC Unit on Anxiety & Stress Disorders, Department of Psychiatry, University of Stellenbosch, University of Cape Town, South Africa (n=393) and the Department of Psychiatry of the University Medical Center Groningen (n=149) (American Psychiatric Association, 1994). These subjects were originally recruited for genetic, phenomenological and treatment response studies. Study participants varied in demographic characteristics and symptom severity across sites adding to the generalizability of the final sample.

***Measures***

The Yale-Brown Obsessive-Compulsive Scale (YBOCS) was used to assess obsessive-compulsive symptoms and their resulting clinical impact or severity (Goodman et al., 1989a, 1989b). The YBOCS consists of a 74-item clinician-rated symptom checklist and a quantitative 40-point severity scale. Obsessive-compulsive symptoms on the YBOCS were coded as 0 (never present) or 1 (ever present). Severity was coded as worst-ever (San Francisco, South Africa) or current (Groningen, Amsterdam, Utrecht, and Boston).

Data on treatment response, defined by a 25% decrease in YBOCS severity score at treatment discharge, were available on 320 subjects from the McLean/Massachusetts General Hospital site who were part of a study on the effectiveness of intensive inpatient treatment for treatment refractory OCD (Stewart et al., 2005). Percent decrease in YBOCS severity scores at the end of treatment was measured as well. Data on comorbid tics were available for 480 subjects from all participating sites except the Utrecht and Groningen sites. Data on family history of OCD or clinically significant obsessive-compulsive symptoms (OCS) were available on 781 subjects. A positive family history was recorded when the subject reported the presence of OC symptoms in at least one family member.

Studies were approved by the Medical Ethical Review Boards of the participating centers. All subjects (and in the case of minors, their parents) gave written informed consent for participation in the study. Children under age 13 years gave assent.

***Statistical Analyses:***

**Latent Class Analyses (LCA).** A series of latent class models from two to seven classes were estimated using 61 YBOCS items in the entire sample of 1611 using MPLUS version 5.2 (Muthén and Muthén, 1998-2006). All YBOCS items were included in the analysis, with the exception of 13 non-specific or open-ended items (e.g., "other contamination obsessions"). To identify the model with best fit, several criteria were considered: the sample-size adjusted Bayesian information criteria (BIC), the size of the smallest class and Lo-Mendell-Rubin (LMR) adjusted likelihood ratio test. BIC is defined by the model and the maximum likelihood estimates of the parameter: BIC = -2 x log (maximum likelihood) + ½ x log (n) x number of independently adjusted parameters within the model (Akaike, 1974). A solution with a relatively small-sized class would not be useful in further analysis and is likely to be an anomaly of the sample. Models were compared using the LMR adjusted likelihood ratio test to identify whether the fit of the model with K classes is better than the fit of a model with K-1 classes (an alternative test, the Vuong-Lo-Mendell-Rubin likelihood ratio, gave nearly identical p-values). We note that as these tests are relatively new,

their performance has not been studied. While not a measure of fit, entropy is an index of the orderliness of the classifications and helps to describe the model. Subjects receive a weight reflecting the probability of belonging to each fitted class so the average of the classification probabilities for each class was also examined. Finally, for the comparisons among the clinical variables, subjects were assigned to their most-likely class to minimize interdependencies.

**Correlations with Clinical Variables.** Associations among the most likely latent class membership derived from the item-level LCA with gender, age of symptom onset, symptom severity as measured by YBOCS severity score, presence of comorbid tics (excluding transient tics), and family history of OCS/OCD were assessed in all available subjects using chi-square analyses, t-tests, and analyses of variance. A p-value $< 0.05$ was considered to be significant.

## Results

### *Demographic and clinical characteristics*

Fifty three percent of the sample was female, and the mean age at assessment was 34 years (SD=12.5, range 4 to 80). The mean age of onset of OC symptoms was 16.7 (SD=9.5, range 0 to 59). Of the 781 patients for whom there were family history data, 37.4% had a family history of OCD/OCS and of the 480 patients for whom there were tic related data, 45% had a comorbid tic disorder.

### *Latent class analysis*

Latent class models were fit estimating two to seven classes per model. As seen in table 1, the BIC values continued to improve as the number of classes increased and the estimated number in each class remained reasonably large, indicators consistent with improved model fit. The diagonal of the matrix of the average latent class probabilities for the most likely class membership by latent class (data not shown) was above .92 in all cases, indicating clear class distinctions. Initially a three-class model appeared to have the best fit, based on the p-values shown in the bottom rows of table 1, although there was also evidence for a five-class model to show a reasonable fit (table 1). A plot of the item endorsement rates for each class in the three-class model clearly showed three groups separated by level of severity. No overlap in symptom endorsement rates was seen among the three classes. This is plotted in Figure 1.

Although the difference in fit between the four-class model and the three-class model was not statistically significant, indicating that the four-class model did not provide a statistically better fit, the change in the p-values from a four- to five-class model was unexpected, and we therefore plotted the five-class solution as well (Figure 2). Three classes (1, 2, and 4) retained the 'level of severity' aspect seen in Figure 1. Class 5 also appeared to reflect severity but at a very low level. Class 3, however, was distinct, showing high endorsement rates on four items: "frequent or excessive hand washing", "fear of contamination from dirt and germs", "excessive or ritualized showering", and "compulsive cleaning of household items". With the exception of these items, class 3 was generally intermediate to low in endorsement rates of the YBOCS-CL items, fitting between classes 4 and 5.

Table 1. Summary of latent class models from 2 through 7 class models.

| **N classes** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|
| BIC-A | 90357.8 | 87461.2 | 86197.5 | 85299.5 | 84684.8 | 84303.5 |
| entropy | .922 | .919 | .902 | .903 | .899 | .905 |
| $N_1$ | 601 | 255 | 426 | 107 | 413 | 117 |
| $N_2$ | 1008 | 720 | 227 | 380 | 233 | 131 |
| $N_3$ | | 666 | 378 | 317 | 238 | 1989 |
| $N_4$ | | | 580 | 373 | 118 | 302 |
| $N_5$ | | | | 434 | 301 | 230 |
| $N_6$ | | | | | 308 | 284 |
| $N_7$ | | | | | | 349 |
| LMR p | <.001 | .001 | .137 | .016 | .686 | .164 |

***Relationship of latent class membership to age of onset and symptom severity***

In examining the 5-class model, with the exception of class 3, all latent classes were significantly inversely associated with age of symptom onset, with the high symptom endorsement classes having a younger mean age of symptom onset than the low symptom endorsement classes (table 2). Despite the wide range of symptom endorsement rates, there was little variation in total YBOCS symptom severity scores between the latent classes, although the trend was for high endorsement classes to be associated with higher YBOCS symptom severity scores (table 2).

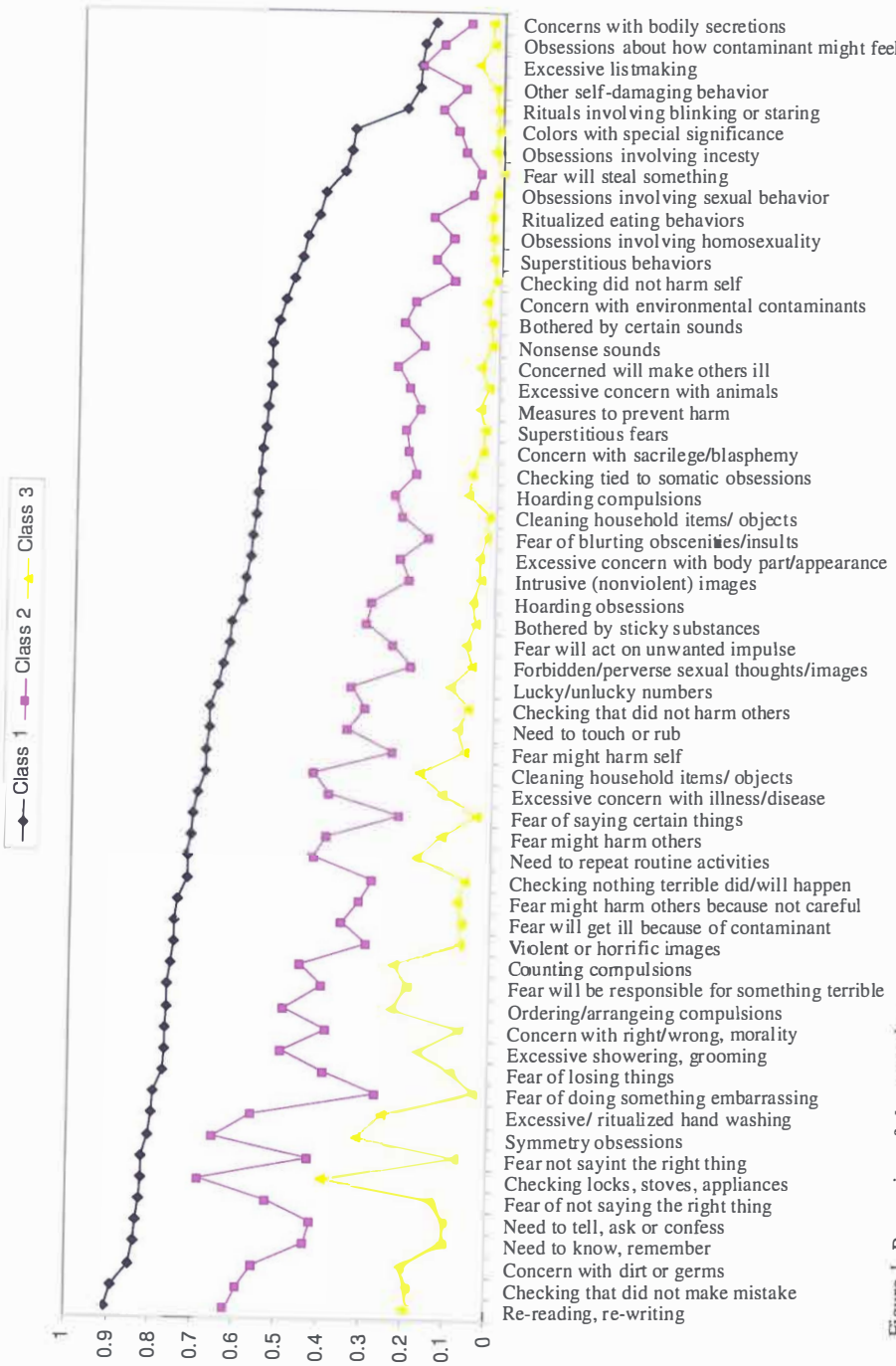


Figure 1. Proportion of class member endorsing each item by most likely class for the 3-class model, sorted by class 1 endorsement rate.

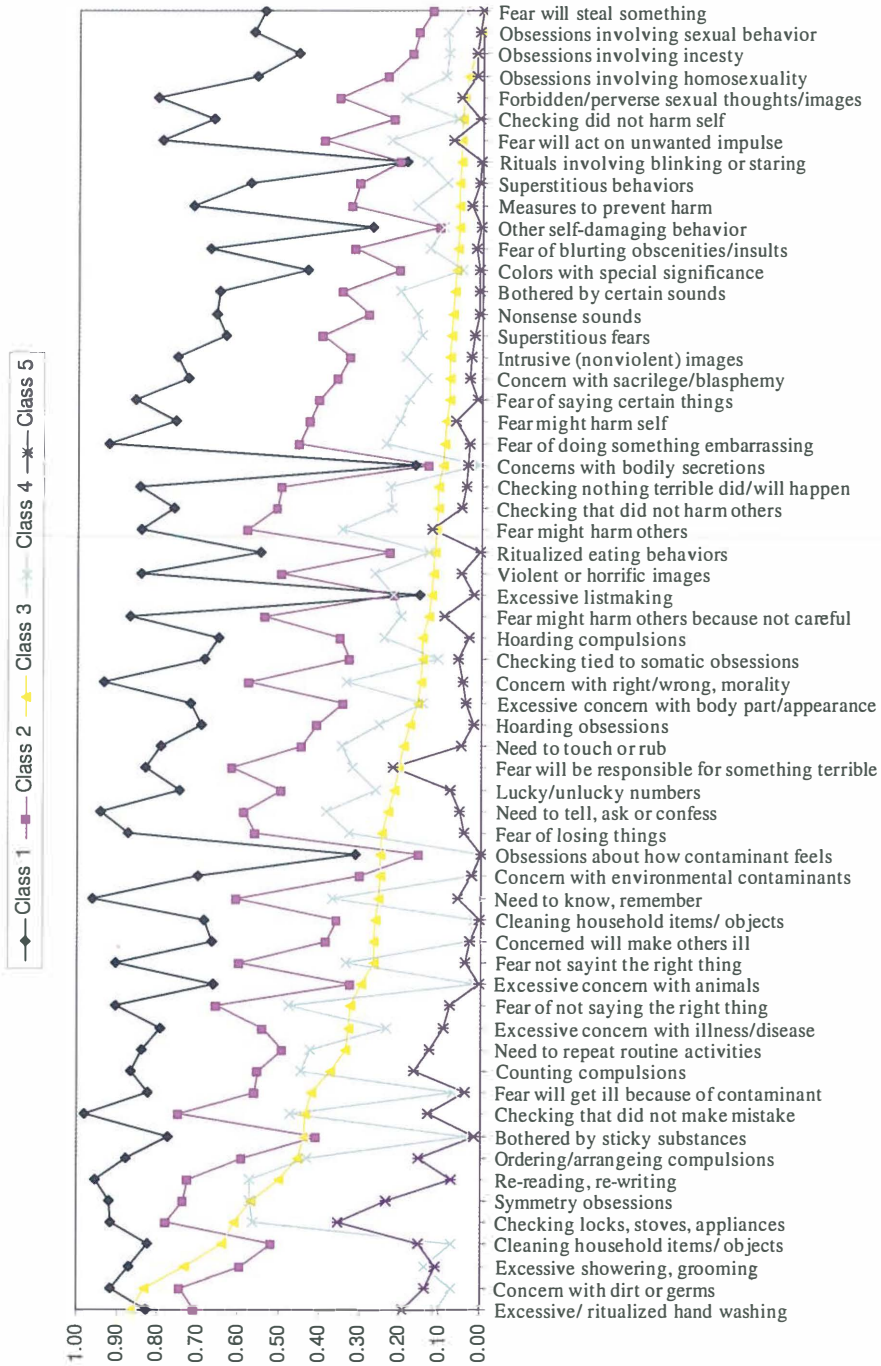


Figure 2. Items endorsement rates by most likely class for the 5-class model.

Table 2. Means (standard deviations) and percentages of clinical and demographic characteristics by latent class.

| | Class 1 | Class 2 | Class 3 (contamination) | Class 4 | Class 5 | Overall | |
|---|---|---|---|---|---|---|---|
| Age of symptom onset N=1440 | 12.5 (7.6) | 14.2 (7.8) | 16.7 (8.4 ) | 15.6 (9.1) | 20.5 (10.7) | F=27.1 | p<0.00001 |
| YBOCS severity score N=543 | 22.6 (6.3) | 21.6 (7.9) | 21.6 (8.6) | 18.8 (8.6) | 19.8 (8.4) | F=2.77 | p=0.027 |
| Age at interview N=1552 | 30.5 (9.6) | 32.9 (11.7) | 34.7 (12.7) | 34.2 (12.7) | 36.2 (13.1) | F=6.45 | p=0.0001 |
| % Female N=1558 | 36.4% | 48.1% | 61.2% | 47.9% | 60.5% | $\chi2$=36.1 | p<0.0001 |
| Tic disorder N=480 | 65.6% | 46.2% | 34.0% | 46.3% | 30.2% | $\chi2$=19.5 | p=0.001 |
| OCD family history N=781 | 40.3% | 38.0% | 34.2% | 42.6% | 35.8% | $\chi2$=2.76 | p=0.599 |

***Relationship of latent class membership to gender, comorbid tics, and family history of OCD/OCS***

Although the proportion of males and females in the overall sample was similar, the ratios of males to females differed between latent classes, with more males than females in the higher symptom endorsement classes (class 1 and 2) and more females than males in the lower symptom endorsement classes (class 4 and 5) (table 2). The relationship between latent class membership and presence of comorbid tics showed that class 1 had proportionally more individuals with comorbid tics, and class 5 had proportionally fewer (table 2). There was no statistically significant relationship between class membership and family history of OCD/OCS.

***Class Membership and Treatment Response***

There was no significant association between treatment response and class membership for any of the outcome variables measured for classes 1, 2, 4, or 5, and the overall test of association between class and treatment outcome was not statistically significant (table 3). However, membership in class 3 was consistently associated with positive treatment response across all outcome measures, and thus we separately examined the relationship between membership in class 3 against membership in any of the other classes with treatment response as defined previously. We found a statistically significant association with all measures of treatment response in these analyses, with the strongest association for decrease in YBOCS compulsion score (5.4 point decrease vs 3.4 point decrease, t=-2.69, p=0.008). In class 3, 63.6% of individuals met criteria for treatment response, as defined by a $\geq$25% improvement in YBOCS total score at the end of treatment, compared with 36.4% of those not in class 3 ($\chi2$=5.63, p = 0.018).

Table 3. Means (standard deviations) and percentages of treatment outcome measures and latent class membership.

| | Class 1 | Class 2 | Class 3 (contamination) | Class 4 | Class 5 | Overall |
|---|---|---|---|---|---|---|
| Decrease in YBOCS total score | 7.0 (6.7) | 6.8 (6.5) | 8.9 (7.9) | 6.6 (6.6) | 6.7 (7.5 ) | F=1.24;p=0.294 |
| % change in YBOCS total score | 25.0 (23.5) | 24.3 (22.6) | 31.9 (27.6) | 26.1 (25.3) | 28.4 (28.9) | F=1.29;p=0.275 |
| % responders | 53.1% | 44.6% | 63.6% | 45.5% | 47.1% | $\chi 2$=6.88;p=0.142 |

**Discussion**

The aim of this study was to elucidate the underlying symptom structure of OCD using latent class analysis, and to relate the resulting classes to clinically relevant subject characteristics. We originally hypothesized that, although LCA uses individuals as the unit of analysis rather than the symptom variables used in factor analysis, the classes derived from LCA would be similar to the symptom categories that have been previously identified using factor analyses, providing further support for the symptom category model. Instead, the results differentiated individuals with OCD into classes based on the frequency with which they endorsed symptoms, rather than by the type of symptoms they endorsed, suggesting an underlying spectrum of OCD severity ranging from uniformly low endorsement rates across symptom subtypes (class 5) to uniformly high endorsement rates across symptom subtypes (class 1). With the exception of class 3 (with predominantly contamination/cleaning symptoms), and contrary to our expectations, there was no evidence of symptom-specific clustering among subjects in our sample, who were recruited from a variety of sites and types of study and represent a broad range of OCD-affected individuals. While this study is the first to use LCA to classify patients based on their symptoms, the findings are consistent with the three previous studies that have utilized an LCA approach to further characterize OCD. Although differing in the numbers of latent classes identified, previous studies found some evidence for an OCD severity spectrum, with the least severe class in each study characterized by few to no psychiatric comorbidities (with or without OCD, depending on the study design), and the most severe class characterized by multiple comorbid conditions (Nestadt et al., 2003; Nestadt et al., 2008). Further, these data are in line with recent epidemiological studies suggesting a common genetic factor underlying OCS, in addition to identifying specific symptom clusters via factor analyses (Mathews et al., 2008; van Grootheest et al., 2008). The fact that the epidemiological studies used variable-based factor analytic approaches rather than person-based latent class analyses suggests that the identification of an OCD spectrum in this study is unlikely to be an artifact of the methodological approach chosen.

In concert with the literature, the more 'severe' classes in our sample were predominantly male, had an earlier age of OC symptom onset, and had a higher rate of comorbid tic disorders when compared to the less 'severe' classes, and there was evidence for a nearly

linear relationship between class membership and these variables (Nestadt et al., 2003, 2008). Previous studies have suggested that particular OCD subtypes may be an alternate expression of a tic disorder, or alternately, that tic disorders and obsessive-compulsive symptoms may have a shared etiology (Bolton et al., 2007; Eapen et al., 1997; Grados et al., 2001). Although we cannot address these hypotheses directly in our dataset, the results of our study do suggest that tics are present at all levels of OCD severity, although they are more common in individuals who manifest high levels of OC symptoms, and therefore may be an expression of disease severity rather than a separate phenomenon, at least in some individuals.

Another interesting finding was the exception to the severity spectrum model of OCD exhibited by class 3, which differed in profile from the other derived latent classes, not being characterized by symptom endorsement rates, as did the classes 1, 2, 4, and 5. Instead it was characterized by high endorsement rates of four specific contamination-related symptoms (64% to 86%). Class 3 appears to represent a separate group of OCD-affected individuals, those with predominantly germ or dirt-related contamination obsessions and the associated cleaning compulsions. This class contained the highest proportion of women of all latent classes, and the second lowest proportion of subjects with tic disorders. It was similar to the low symptom endorsement classes (4 and 5) in that individuals in this class had a comparatively late age of symptom onset, and although not statistically significant, the lowest proportion of individuals with a family history of OCD/OCS. Most interestingly, however, individuals in this class were more likely to respond to treatment than were individuals in the other four classes, including the low endorsement classes, suggesting that this class may be not only phenomenologically distinct, but may also have different etiologies and more favorable treatment outcome than the other classes.

The principal limitation of this study is that it utilizes data collected from multiple sources using a variety of ascertainment and assessment techniques, resulting in variation in the demographic and clinical characteristics of the subjects, as well as in incomplete data for some variables. Although it is possible that this variation results in decreased precision of the findings, the heterogeneity of the sample has the important advantage of improving the generalizability of the findings. The differences in data collection approaches resulted in variation in the age of individuals in the sample across sites, introducing the possibility of recall bias. For example, seen in table 2, the age at interview increased with increasing latent class, suggesting that the older the age of the subject, the lower the severity. This could be a reflection of the natural course of OCD, where a substantial proportion of individuals have a decrease in symptomatology with increasing age (Angst et al., 2004). However, it could also be a manifestation of recall bias, with younger individuals more accurately assessing their lifetime severity, and leading to a false association between age at

interview and latent class. Similarly, we did not have data on the presence of tic disorders or family history of OCD/OCS in the entire sample, potentially introducing a bias. Similarly, the treatment response data were collected at a single site (MGH) in a subset of patients with severe, refractory OCD who may not be representative of OCD patients in general. Finally, we assigned the study participants to the most likely latent class, necessitated by the lack of full data on all covariates. While the mean probability of assignment to class was very high, it is possible that the significance associated with the differences among classes shown in tables 2 and 3 may be sub-optimal.

In summary, the results of this first latent class analysis of YBOCS symptoms conducted to date in a large group of OCD patients provides compelling evidence for OCD defined as a single spectrum based on severity or symptom endorsement rates rather than, or perhaps in addition to, the distinct subtypes of symptoms that have been identified via factor analysis, and for a separate, less severe class, characterized by predominantly cleaning/contamination symptoms, a later age of onset, and good treatment response. These findings, with the exception of class 3, are consistent with our previous genetic epidemiology studies suggesting the presence of an underlying latent susceptibility that predisposes to the development of OCD, regardless of symptom subtype, and has a genetic etiology (Mathews et al., 2008; Mathews et al., 2007b; van Grootheest et al., 2008).

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# Part 2

## Candidate gene studies in obsessive compulsive disorder and Tourette syndrome

# The Role of the BDNF Val66Met Polymorphism in the Phenotypic Expression of Obsessive-Compulsive Disorder

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**Abstract**

*Evidence suggests that the Val66Met variant of the brain-derived neurotrophic factor (BDNF) gene may play a role in the etiology of Obsessive-Compulsive Disorder (OCD). In this study, the role of the BDNF Val66Met variant in the etiology and the phenotypic expression of OCD is investigated. Associations between the BDNF Val66Met variant and OCD, obsessive-compulsive symptom dimensions, Yale-Brown Obsessive Compulsive Scale (YBOCS) severity scores, age of onset and family history of obsessive-compulsive symptoms were assessed. The BDNF Val66Met variant was genotyped in 419 patients with sub-/clinical OCD and 650 controls. No differences in allele or genotype frequency were observed between cases and controls. In females with OCD, the Met66Met genotype was associated with later age of onset and a trend for a negative family history, whereas the Val66Val genotype was associated with a trend for lower YBOCS severity scores. Item-level factor analysis revealed six factors: 1) Contamination/cleaning; 2) Aggressive obsessions/checking; 3) Symmetry obsessions, counting, ordering and repeating; 4) Sexual/religious obsessions; 5) Hoarding and 6) Somatic obsessions/checking. A trend was found for a positive association between Factor 4 (sexual/religious) and the BDNF Val66Val genotype. The results suggest that BDNF function may be implicated in the mediation of OCD. We found that for the BDNF Met66Met genotype may be associated with a milder phenotype in females and a possible role for the BDNF Val66Val genotype and the BDNF Val66 allele in the sexual/religious obsessions.*

## Introduction

Obsessive-compulsive disorder (OCD) is both clinically and genetically heterogeneous. This heterogeneity obscures the findings of clinical, natural, and treatment response studies. Family and twin studies indicate that genetic factors play an important role in mediating at least some forms of OCD (Hettema et al., 2001; Nestadt et al., 2002; Pauls and Alsobrook, 1999; van Grootheest et al., 2005). Although major advances have been made in the last decade in characterizing the phenomenology and psychobiology of OCD, its phenotypic and genetic heterogeneity has complicated the search for vulnerability genes.

One approach to deal with this heterogeneity is to classify OCD according to clinically defined characteristics and to search for the genetic underpinnings of these possibly more homogeneous phenotypes. Most studies that focused on the phenomenology of obsessive-compulsive (OC) symptoms used the Yale-Brown Obsessive-Compulsive Scale symptom checklist (YBOCS-CL), a screening instrument for the presence of frequently encountered OC symptoms (Goodman et al., 1989a, 1989b). Factor analytic studies using predefined symptom categories of the YBOCS-CL have consistently found four OC symptom dimensions, namely (1) aggressive, sexual, somatic and religious obsessions with checking obsessions; (2) symmetry obsessions with ordering and arranging compulsions; (3) contamination obsessions and cleaning compulsions, and (4) hoarding obsessions and compulsions (Miguel et al., 2005). These symptom dimensions are hypothesized to represent more homogeneous genetic dimensions of OCD (Miguel et al., 2005). The YBOCS-CL symptom dimension of aggressive/sexual obsessions and checking compulsions, and the symptom dimension of symmetry obsessions and ordering and arranging compulsions have been found to be more familial than the other symptom dimensions (Alsobrook et al., 1999). A recent family study showed statistically significant correlations of scores on all symptom dimensions between affected sib pairs (Hasler et al., 2007). Moreover, a segregation analysis of the symptom dimensions in siblings with Tourette syndrome (Leonard et al., 1993), a putative OCD spectrum disorder, suggested a familial component for the symmetry, ordering and counting factor as well as the aggression/sexual obsessions factor (Leckman et al., 2003). Furthermore, two recent genome scans in which hoarding was used as a phenotype found suggestive linkage to regions on chromosomes 14, 4q, 5q, and 17q (Samuels et al., 2007; Zhang et al., 2002). A recent candidate gene study recently found the "obsessional/checking" OCD symptom subtype to be associated with earlier age of OCD onset and the *Met158Met* genotype of the *COMT Val158Met* polymorphism (Lochner et al., 2008). Three studies investigated the relation between symptom dimensions and an insertion/deletion polymorphism in the serotonin transporter gene (*5HTTP*) (Cavallini et al., 2002; Hasler et al., 2006; Kim et al.,

2005). Two of these studies found a trend for a positive association between a symptom dimension with counting and repeating rituals (Cavallini et al., 2002; Hasler et al., 2006). The third study found an association between this polymorphism and a factor with religious and somatic obsessions (Kim et al., 2005). Two other studies found no association between symptom dimensions and the *Val66Met* polymorphism in the Brain Derived Neurotrophic factor gene (*BDNF*) (Alonso et al., 2008a; Wendland et al., 2007). Taken together, these studies suggest that OC symptom dimensions comprise distinct genetic entities, which would justify using them as phenotypes in molecular genetic studies of OCD. Since the symptom dimensions obtained by factor analysis can be quantified as factor scores for each patient, these symptoms dimensions are statistically more powerful than the investigation of OCD as a dichotomous diagnostic entity (Silverman and Palmer, 2000).

The *BDNF* gene is an attractive OCD candidate gene, from both a brain development and neurotransmitter perspective. *BDNF* has been implicated in neuronal survival and in activity-dependent neuroplasticity (Hennigan et al., 2007). Studies in knockout mice as well as those using B cell lines suggest that *BDNF* modulates the serotonin transporter function (Daws et al., 2007; Mössner et al., 2000).

A polymorphism in the *BDNF* gene that causes a valine to methionine substitution in the prodomain of the BDNF protein (*Val66Met*) has been shown to reduce activity-dependent BDNF secretion in transfected neurons (Egan et al., 2003). Studies in *BDNF Met66Met* mice suggest that this polymorphism may be implicated in anxiety-related behaviors (Chen et al., 2006). Pharmacological and neurobiological studies suggest that the serotonergic system is involved in the pathogenesis of OCD (Westenberg et al., 2007). In summary, there are several lines of evidence indicating that the serotonergic system and, more specifically *BDNF*, may be implicated in the etiology of OCD.

Thus far, seven studies have reported on the *BDNF Val66Met* variant in OCD, using either a family based approach (Dickel et al., 2007; Hall et al., 2003; Mössner et al., 2005; Zai et al., 2005b), or a case-control design (Alonso et al., 2008a; Hemmings et al., 2008; Wendland et al., 2007). These studies are summarized in table 1. Findings have been inconsistent. In the family based studies, an ***under*** transmission of the *BDNF Met66* allele was found in one study of patients with childhood-onset OCD (Hall et al., 2003), whereas the other studies did not show a preferential transmission of either of the alleles. In the case-control studies an association was found of the *BDNF Met66* allele with OCD in males (Hemmings et al., 2008) whereas Wendland et al. (2007) did not find an association. One family based study using haplotype analysis found a haplotype marked by the *BDNF Met66* allele to be undertransmitted and therefore likely to confer a protective effect against OCD (Hall et al., 2003), whereas another study found a haplotype including the *BDNF Val66* allele to be associated with a reduced risk for OCD (Alonso et al., 2008a). Some studies

also investigated the association of the *BDNF Val66Met* genotype and dimensional OC phenotypes such as symptom dimensions, age of onset and symptom severity (Alonso et al., 2008a; Hall et al., 2003; Wendland et al., 2007; Zai et al., 2005). One study found an association of the *BDNF Met66* allele with earlier age of onset in males and the *BDNF Val66Met* genotype with increased severity in women (Hemmings et al., 2008), whereas other studies did not find an association with these dimensional phenotypes. Similarly, a family based study of the *BDNF Val66Met* in patients with TS (with or without comorbid OCD), a disorder genetically related to OCD, found no association between TS and the *BDNF Val66Met* polymorphism (Klaffke et al., 2006).

An important drawback of the studies mentioned concerns the relatively small sample sizes, which possibly explains the inconsistent findings to date. In this study, trying to overcome this disadvantage, we investigated the *BDNF Val66Met* polymorphism in a large group of patients with OCD. The aims of our study were: (1) to replicate the association of the *BDNF Val66Met* polymorphism and OCD in patients with early-onset OCD and/or males described previously (Hall et al., 2003; Hemmings et al., 2008) (2) to investigate the association of this polymorphism with specific OC symptom dimensions, age of onset of OC symptoms (irrespective of the nature thereof), OC symptom severity and family history of OC symptoms. Since sex differences have been described in both clinical and genetic studies of OCD (Labad et al., 2008; Lochner et al., 2004), we also investigated sex-specific associations of this polymorphism with clinical characteristics of OCD.

This project encompasses a joint venture between the Department of Psychiatry of VU University Medical Center (VUMC) and the Department of Psychiatry of the University Medical Center Groningen (UMCG) in the Netherlands and the MRC Research Unit on Anxiety Disorders, in collaboration with the MRC/US Centre for Molecular and Cellular Biology, University of Stellenbosch in South Africa. The study was approved by the Medical Ethical Review Boards of the participating centers. All patients (and in case of minors, their parents) gave written informed consent for participation in the study.

Table 1. Association studies investigating the *BDNF Val66Met* Variant in Obsessive-Compulsive Disorder and Tourette Syndrome.

| Study | Population | n | Diagnostic criteria | Diagnosis | Test | Val66 | Met66 | p |
|---|---|---|---|---|---|---|---|---|
| Family-based studies | | | | | | | | |
| Hall et al. 2003[a] | USA | 164 | DSM-IV | OCD | TDT | 58 | 26 | 0.0005 |
| Mössner et al. 2005 | German | 67 | DSM-IV | OCD without TS | TDT | 17 | 20 | 0.62 |
| Zai et al. 2005b[a] | Canada | 152 | DSM-IV | OCD | FBAT | | | 0.587 |
| Dickel et al. 2007[b] | USA | 54 | DSM-III-R | OCD±TS | TDT | 15 | 15 | 1.00 |
| Klaffke et al. 2006[a] | German | 88 | DSM-III-R | TS±OCD | ETDT | 24 | 30 | 0.414 |

| Study | Population | n | Diagnostic criteria | Diagnosis | Test | | Genotype counts Val/Val | Val/Met | Met/Met | p-values Allelic | Genotypic |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Case-control studies | | | | | | | | | | | |
| Wendland et al. 2007 | Caucasian | 295 | DSM-IV | OCD±tics | $x^2$/exact | | | | | | |
| | | | | | | Patients | 192 | 92 | 11 | | |
| | | | | | | Controls | 428 | 206 | 23 | 0.950 | 1.000 |
| | | | | | | Male patients | 82 | 37 | 5 | | |
| | | | | | | Male controls | 193 | 98 | 13 | 0.715 | 0.876 |
| | | | | | | Female patients | 110 | 55 | 6 | | |
| | | | | | | Female controls | 235 | 108 | 10 | 0.612 | 0.844 |
| Hemmings et al. 2008 | Afrikaner | 112 | DSM-IV | OCD±tics | $\chi 2$ | Patients | 73 | 33 | 6 | | |
| | | | | | | Controls | 95 | 43 | 2 | 0.355 | 0.249 |
| | | | | | | Male patients | 33 | 19 | 5 | | |
| | | | | | | Male controls | 25 | 8 | 0 | 0.036 | 0.117 |
| | | | | | | Female patients | 40 | 14 | 1 | | |
| | | | | | | Female controls | 70 | 35 | 2 | 0.438 | 0.717 |
| Alonso et al. 2008a[b] | Spanish | 115 | DSM-IV | OCD±tics | Logistic regression under dominant, recessive, additive, codominant and overdominant model | | | | | n.s. | n.s. |

OCD, Obsessive-compulsive disorder; TS, Tourette syndrome; TDT, Transmission Disequilibrium Test; FBAT, Family Based Association Test; ETDT, Extended Transmission Disequilibrium Test. [a] Data on analyses stratified by sex are not provided. [b] Results of analyses after stratification by sex were insignificant.[c] Part of the subjects in this study are included in the current analyses.

**Participants**

Patients at the MRC Unit on Anxiety and Stress disorders of the University of Stellenbosch were recruited by physician referral, media advertisements, the Mental Health Information Centre (MHIC) and the OCD Association of South Africa (OCDSA), as described previously (Hemmings et al., 2008). Patients at the outpatient Clinic for Anxiety Disorders, GGZ Buitenamstel in Amsterdam and the University Medical Center in Groningen (The Netherlands) were recruited by physician referral, media advertisements and patient societies for patients with anxiety disorders.

All patients met criteria for either a lifetime (Netherlands) or a current (South Africa) diagnosis of OCD or subclinical OCD according to DSM-IV criteria (American Psychiatric Association, 1994). Diagnoses were established using the Structured Clinical Interview for Axis I disorders (SCID-I/P) (First et al., 1998) or the Mini International Neuropsychiatric Interview (MINI) version 5.0.0. (Sheehan et al., 1998). Subclinical OCD was defined as OC symptoms that are either time-consuming (i.e., take 1 hr a day or more, but without causing distress) or interference or that are distressing or causing interference, but take less than 1 hr a day. Only Caucasian patients and controls from the South African site were included; patients from the Netherlands were from the general Dutch population. Control subjects from both South Africa and the Netherlands encompassed an unscreened convenience sample recruited from the general population. The study included 606 phenotyped patients of whom 419 (n=220 from the Netherlands, n=199 from South Africa) were also genotyped for the *BDNF Val66Met* polymorphism. YBOCS-CL data were available for 579 patients. Further, genotype data were available of 650 controls (n=535 Dutch, n=115 Caucasian South African). Some of the subjects from the South African cohort were included in a previous case-control study (Hemmings et al., 2008).

Measurements

The YBOCS-CL was used to assess OC symptom characteristics (Goodman et al., 1989a, 1989b). In some patients from Amsterdam (n=111) an extended 80-item self-response version of the YBOCS was used. This 80-item self-report YBOCS-CL was translated from the version used in the TSA genetics consortium on Tourette's disorder and within the scope of the OCF international collaboration on the genetics of OCD. In a comparison of an interview- versus self-report version of the YBOCS-CL in the US, the self-report version showed good internal consistency and test-retest reliability and strong convergent validity with the interview version (Steketee et al., 1996).

Current severity of the obsessive-compulsive symptoms was assessed using the YBOCS Severity Scale (YBOCS-SS) (Goodman et al., 1989, 1989b). In contrast to YBOCS-symptom checklist data, we used the original interview-based severity scale as the YBOCS

severity scale. In addition, age of onset of OC symptoms and family history of OC symptoms were determined. Family history was considered to be positive if the proband reported the presence of recognizable OC symptomatology that bothered the person in at least one first-degree family member.

Genotyping

DNA was isolated from blood using a chloroform/isopropanol extraction (Meulenbelt et al., 1995), from buccal swabs using a salting out procedure (Miller et al., 1988) or from sputum using an Oragene DNA self collection kit (DNA Genotek, Inc., Ottawa, Canada) according to manufacturers instructions.

All DNA samples from the South African cohort were obtained from blood. Genotyping of the South African cohort was performed by allele-specific restriction enzyme digestion of a PCR product with *Nla*III, as described previously (Hemmings et al., 2008). Complete digestion was taken to be the presence of the consecutive 57 bp band.

Dutch samples were genotyped in a SNPlex™ genotyping assay (Applied Biosystems, Foster City, CA) or by Taqman genotyping assay (Applied Biosystems) (Assay on demand, ID CD_11592758_10) according to manufacturer's instructions.

All controls from the Dutch cohort were genotyped in a SNPlex™ genotyping assay: 104 blood samples with a 100% success rate and 450 buccal swab samples with 95.7% success rate. The genotypes of the Dutch patients were determined in two SNPlex™ runs with a mean success rate of 90.2% and a Taqman run with a success rate of 94.7%.

Statistical Analysis

Data from the South African and Dutch cohorts were combined. Power calculations were performed with the genetic power calculator (Purcell et al., 2003) assuming a disease prevalence of 2% (Angst et al., 2004; Weissman et al., 1994). Hardy-Weinberg equilibrium was tested using chi square tests using Microsoft Office Excel. Genotype and allele frequencies were compared between patients and controls and between patients with different phenotypes using Fisher's exact tests. Since data on continuous variables such as age of onset of OC symptoms, YBOCS-SS score and factor scores showed a skewed distribution, differences in these variables between patients with different genotypes and between the *BDNF Val66* and the *Met66* allele were examined using non-parametric tests (Kruskal-Wallis and Mann-Whitney *U* tests).

Factor Analyses

Different versions of the YBOCS-CL were used with some non-overlapping items. Only items overlapping with the 74-item YBOCS-CL version described by Goodman et al. were

used in the analysis (Goodman et al., 1989a, 1989b). The extended 80-item self-report version of YBOCS-CL used for some Dutch patients, contained several items concerning obsessions with symmetry and exactness. However, since no distinction was made with respect to magical thinking accompanying these obsessions, one item was created coding for any symmetry obsession in the patients. Moreover, in the 80-item self report version of the YBOCS-CL seven items were not available (as indicated in table 4). Missing values in the YBOCS-CL and YBOCS-SS were imputed using Solas™ 3.2 (Statistical Solutions, Ltd, Cork, Ireland), using predictive model-based imputation. For the YBOCS-CL only the items with missing values were included in the imputation model whereas for the YBOCS-SS all items were used in the imputation model. Five different datasets were imputed according to recommendations by Schafer (Schafer, 1999). Items from the "miscellaneous" or "other" categories were omitted from the analysis since these symptoms were considered to be too heterogeneous.

Explorative item-by-item level factor analysis with promax rotation was performed using MPlus (Muthén and Muthén, 1998-2006). Promax rotation was used, since this form of rotation allows the factors to be correlated. Subsequently, confirmatory factor analysis for categorical variables was performed to establish the number of factors and factor constitution using the following fit indices: the $\chi 2$ statistic, the comparative fit index (CFI), the Tucker-Lewis Index (TLI), the Root Mean Square Error of Approximation (RMSEA) and the Standardized Root Mean Square Residual (SRMR). Values of the CFI and of TLI approaching 0.95, values approaching 0.08 of the SRMR and values of the RMSEA $< 0.05$ are generally indicative of a good fit (Browne and Cudek, 1993; Hu and Bentler, 1999).

For the best fitting model, mean score per item for each of the obtained factors was calculated for each patient, representing the prominence of this factor in the patient. Mean results for the five imputed datasets are reported. Kruskal-Wallis tests and Mann-Whitney *U* tests were performed to investigate whether mean item score per factor were associated with the *BDNF Val66Met* genotype or alleles. Kruskal-Wallis tests and Mann-Whitney *U* tests were performed using R statistical package (R Development Core Team, 2008). For these tests empirical p-values were calculated based on 1000 random permutations. This was done for the entire sample and for male and female patients separately in order to obtain robust p-values for each gender. Ten phenotypes were tested (OCD as a dichotomous trait, age of onset of OC symptoms, YBOCS severity score, family history and 6 symptom dimensions). These phenotypes were tested for the entire sample and for males and females separately. Because the total sample is not independent from the two genders, a p-value of $0.05/20 = 0.0025$ was considered significant for these tests. All statistical tests were performed using the Software Package for Social Sciences (SPSS) version 14.0 (SPSS, Inc, Chicago, IL) except noted otherwise.

**Sample Size and Power Considerations**

Since large samples produce more stable variable loadings across repeated sampling and more precise estimates of population loadings (MacCallum et al., 1999), we included non-genotyped participants in the factor analysis as well.

Similarly, we included previously studied Caucasian subjects from South Africa in our current association study to increase sample size and power. Based on the allele frequency in our combined control population, the power to detect a dominant effect of the *BDNF Met66* allele with a two times increased risk for OCD in the *BDNF Val66Met* and *Met66Met* genotypes compared to the *Val66Val* genotype in the case-control study was 99.9%, 92.0%, and 95.8% in the total sample and males and females separately, respectively. The power to detect a recessive effect of the *BNDF Met66* allele with a two times increased risk for OCD with the *BDNF Met66Met* genotype compared to the *BDNF Val66Met* and *Val66Val* genotypes was 34.0% in the total sample, 17.3% in males, and 21.6% in females.

Demographic and clinical characteristics of the patients studied are summarized in table 2. The Dutch patients were significantly older than the South African patients ($p<0.001$). The Dutch phenotyped group contained significantly more women ($p<0.012$) than the South African group, although this difference did not reach significance in the genotyped patients.

Table 2. Demographic and clinical characteristics of the OCD patients in the different cohorts.

| | Total cohort | South African cohort | Dutch cohort | p |
|---|---|---|---|---|
| All patients | | | | |
| n | 606 | 199 | 407 | |
| Age (y, mean±SD) | 35.4±12.7 | 32.8±14.5 | 36.7±11.6 | <0.001 |
| Female (%) | 334 (55.1%) | 95 (47.7%) | 239 (58.7%) | 0.011 |
| Age of onset (y, mean±SD) | 17.4±9.9 | 17.2±10.9 (n=188) | 17.5±9.3 (n=366) | 0.134 |
| YBOCS severity (mean±SD) | 20.5±8.1 | 19.9±7.6 (n=195) | 20.7±8.3 (n=392) | 0.137 |
| Positive family history of OC behavior (%) | | | 94 (44.3%) (n=212) | |
| Genotyped patients | | | | |
| n | 419 | 199 | 220 | |
| Age (y, mean±SD) | 35.3±13.4 | 32.8±14.5 | 37.5±11.9 | <0.001 |
| Female (%) | 219 (52.3%) | 95 (47.7%) | 124 (56.4%) | 0.079 |
| Age of onset (y, mean±SD) | 17.0±10.2 | 17.2±10.9 (n=188) | 16.7±9.5 (n=192) | 0.648 |
| YBOCS severity (mean±SD) | 20.0±7.9 | 19.9±7.6 (n=195) | 20.0±8.2 | 0.659 |
| Positive family history of OC behavior (%) | | | 64 (50.8) (n=126) | |

Case-Control Study

Genotype and allele frequencies, as well as case-control study results are summarized in table 3. Genotype distribution was in Hardy-Weinberg equilibrium for all groups. There was no significant difference in genotype (p=1.000) or allele frequency (p=0.926) between the South African and Dutch control groups, nor were there differences in genotype or allele frequency between male and female controls from South Africa and from the Netherlands respectively (genotypes: p=0.834 in males, and p=0.920 in males; alleles: p=0.472 in males, p=0.740 in females). Therefore, these groups were pooled for the subsequent analyses. There was a trend for an association between the *BDNF Met66* allele and OCD in female Dutch patients (p=0.025). This was not found in the South African sample or in the pooled sample.

Factor Analysis

YBOCS-CL data were available of 579 OCD patients. In the exploratory factor analysis, thirteen factors with an eigenvalue>1 were identified. Forty-three YBOCS-CL items were included in the factor analysis, with the number of participants being higher than the recommended 5-10 subjects per item. Based on the results from the iterative exploratory factor analyses, we performed confirmatory factor analyses to obtain a more parsimonious model. The fit indices of the one to six factor models are summarized in IV. The one to six factor models had an increasingly better fit. Since the seven factor model did not converge, models with 7 or more factors were not investigated.

The six factor model showed the best fit and fulfilled the criteria for satisfactory model fit and was therefore used in subsequent analyses. Factor loadings for this model are shown in table 5. This model had the following factors:

Factor 1: Contamination obsessions and cleaning compulsions; Factor 2: Aggressive obsessions and checking; Factor 3: Symmetry obsessions, ordering, arranging, counting, and repeating compulsions; Factor 4: Sexual and religious obsessions; Factor 5: Hoarding obsessions and compulsions; and Factor 6: Somatic obsessions and checking.

Pearson's correlations between mean scores per item for the different factors and Cronbach's alpha for internal consistency of the different factors are summarized in table 6. Although mean scores per item for the different factors were significantly correlated (data not shown), the correlations between them were relatively low.

Table 3. Genotype and allele frequencies.*

| | *Val66Val* | *Val66Met* | *Met66Met* | n | *p*-value | *Val66* | *Met66* | *p*-value |
|---|---|---|---|---|---|---|---|---|
| Total cohort | | | | | | | | |
| OCD | 260 (62.1%) | 137 (32.7%) | 22 (5.3%) | 419 | | 657 (78.4%) | 181 (21.6%) | |
| Control | 428 (65.8%) | 202 (31.1%) | 20 (3.1%) | 650 | p=0.149 | 1058 (81.4%) | 242 (18.6%) | p=0.095 |
| Male OCD | 125 (62.5%) | 65 (32.5%) | 10 (5.0%) | 200 | | 315 (78.8%) | 85 (21.3%) | |
| Male control | 183 (65.8%) | 89 (32.0%) | 6 (2.2%) | 278 | p=0.234 | 455 (81.8%) | 101 (18.2%) | p=0.247 |
| Female OCD | 135 (61.6%) | 72 (32.9%) | 12 (5.5%) | 219 | | 342 (78.1%) | 96 (21.9%) | |
| Female control | 245 (65.9%) | 113 (30.4%) | 14 (3.8%) | 372 | p=0.436 | 603 (81.0%) | 141 (19.0%) | p=0.229 |
| South African cohort | | | | | | | | |
| OCD | 128 (64.3%) | 62 (31.2%) | 9 (4.5%) | 199 | | 318 (79.9%) | 80 (20.1%) | |
| Control | 76 (66.1%) | 36 (31.3%) | 3 (2.6%) | 115 | p=0.784 | 188 (81.7%) | 42 (18.3%) | p=0.602 |
| Male OCD | 63 (60.6%) | 34 (32.7%) | 7 (6.7%) | 104 | | 160 (76.9%) | 48 (23.1%) | |
| Male control | 21 (72.4%) | 8 (27.6%) | 0 (0.0%) | 29 | p=0.350 | 50 (86.2%) | 8 (13.8%) | p=0.147 |
| Female OCD | 65 (68.4%) | 28 (29.5%) | 2 (2.1%) | 95 | | 158 (83.2%) | 32 (16.8%) | |
| Female control | 55 (64.0%) | 28 (32.6%) | 3 (3.5%) | 86 | p=0.730 | 138 (80.2%) | 34 (19.8%) | p=0.498 |
| Dutch cohort | | | | | | | | |
| OCD | 132 (60.0%) | 75 (34.1%) | 13 (5.9%) | 220 | | 339 (77.0%) | 101 (23.0%) | |
| Control | 352 (65.8%) | 166 (31.0%) | 17 (3.2%) | 535 | p=0.123 | 870 (81.3%) | 200 (18.7%) | p=0.065 |
| Male OCD | 62 (64.6%) | 31 (32.3%) | 3 (3.1%) | 96 | | 155 (80.7%) | 37 (19.3%) | |
| Male control | 162 (65.1%) | 81 (32.5%) | 6 (2.4%) | 249 | p=0.887 | 405 (81.3%) | 93 (18.7%) | p=0.914 |
| Female OCD | 70 (56.5%) | 44 (35.5%) | 10 (8.1%) | 124 | | 184 (74.2%) | 64 (25.8%) | |
| Female control | 190 (66.4%) | 85 (29.7%) | 11 (3.8%) | 286 | p=0.071 | 465 (81.3%) | 107 (18.7%) | p=**0.025** |

*$P$ values <0.0025 were considered significant; $P$ values below the nominal significance level of 0.05 are indicated in bold.

Table 4. Goodness of fit indices obtained by confirmatory factor analysis.*

| Fit index | 1 factor model | 2-factor model | 3-factor model | 4-factor model |
|---|---|---|---|---|
| CFI | 0.506 | 0.715 | 0.845 | 0.853 |
| TLI | 0.650 | 0.802 | 0.891 | 0.897 |
| RMSEA | 0.110 | 0.083 | 0.062 | 0.060 |
| SRMR | 0.177 | 0.144 | 0.119 | 0.117 |

CFI, Comparative fit index; TLI, Tucker-Lewis Index; RMSEA, Root Mean Square Error of Approximation; SRMR, Standardized Root Mean Square Residual.

* Values of the CFI and of TLI approaching 0.95, values approaching 0.08 of the SRMR and values of the RMSEA < 0.05 are generally indicative of a good fit.

Table 6. Mean Cronbach's alpha for the different factors and Pearson's correlations for the mean score per item between the different factors

| | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 |
|---|---|---|---|---|---|---|
| Cronbach's alpha | 0.844 | 0.786 | 0.688 | 0.669 | 0.829 | 0.630 |
| Factor 2 | 0.339 | 1.000 | | | | |
| Factor 3 | 0.263 | 0.421 | 1.000 | | | |
| Factor 4 | 0.156 | 0.466 | 0.148 | 1.000 | | |
| Factor 5 | 0.143 | 0.197 | 0.219 | 0.128 | 1.000 | |
| Factor 6 | 0.500 | 0.416 | 0.199 | 0.225 | 0.126 | 1.000 |

**Genotype-Phenotype Correlations**

Both genotype data and mean scores per item for the different factors were available of 392 patients with OCD. The results of the Kruskal-Wallis and Mann-Whitney *U* tests comparing mean scores per item for the different factors between patients with different genotypes are summarized in table 7. None of the factors showed an association with *BDNF Val66Met* genotype after correction for multiple-testing. However, there was a trend for a positive association between factor 4 (Sexual and religious obsessions) and the *BDNF Val66Val* genotype in the patients.

The results of the comparisons of age of onset of OC symptoms and OCD severity by *BDNF Val66Met* genotype are summarized in table 8. There was a significant sex effect with respect to age of onset of OC symptoms and genotype; age of onset of OC symptoms in women with the *BDNF Met66Met* genotype was significantly later than in the other groups (p=0.002). Further, there was a trend towards lower OCD severity in female patients with the *BDNF Val66Met* or *Met66Met* genotypes compared to female patients with the *BDNF Val66Val* genotype (p=0.023).

Table 5. Mean factor loadings for the five imputed datasets obtained by confirmatory factor analysis.*

| | |
|---|---|
| **Factor 1: Contamination and cleaning** | |
| Concern with dirt or germs | 1.000 |
| Excessive or ritualized hand washing[a] | 0.851 |
| Concerns or disgust with bodily waste or secretion (e.g., urine, faeces, and saliva) | 0.772 |
| Excessive concern with household items (e.g., cleaners, solvents)[a] | 0.758 |
| Excessive concern with environmental contaminants (e.g., asbestos, radiation, toxic waste) | 0.743 |
| Bothered by sticky substances or residues | 0.712 |
| Compulsions involving cleaning of household items or other inanimate objects | 0.705 |
| Other measures to prevent or remove contact with contaminants | 0.696 |
| Excessive or ritualized showering, bathing, teeth brushing, grooming, or toilet routine | 0.684 |
| Excessive concern with animals (e.g., insects) | 0.676 |
| *Concerned that I will get ill because of contaminant* | 0.481 |
| *Concerned that I will get others ill by spreading contamination (aggressive)*[a] | 0.466 |
| **Factor 2: Aggressive obsessions and checking** | |
| Fear that I will harm others because of not being careful enough (e.g., hit/run MVA) | 1.000 |
| Fear that I will steal things | 0.738 |
| Fear that I might harm others | 0.720 |
| Checking that **I** did not/will not harm others | 0.707 |
| Fear that I will act on unwanted impulses (e.g., to stab a friend) | 0.696 |
| Fear that I will be responsible for something else terrible happening (e.g., fire, burglary) | 0.691 |
| Fear of blurting out obscenities or insults | 0.694 |
| Checking that nothing terrible did/will happen | 0.655 |
| Fear of doing something else embarrassing | 0.606 |
| Fear that I might harm myself | 0.537 |
| *Violent or horrific images* | 0.408 |
| *Checking that I did not/will not harm self* | 0.430 |
| *Checking that I did not make a mistake* | 0.428 |
| *Concerned that I will get others ill by spreading contamination (aggressive)*[a] | 0.361 |
| *Checking locks, stove, appliances, etc.* | 0.359 |
| **Factor 3: Symmetry obsessions, ordering, arranging, counting, and repeating compulsions** | |
| Ordering/arranging compulsions | 1.000 |
| Re-reading or re-writing | 0.685 |
| Counting compulsions | 0.643 |
| Symmetry obsessions | 0.634 |
| Need to repeat routine activities (e.g., in/out door, up/down from chair, i.e., repeating rituals) | 0.537 |
| *Checking that I did not make a mistake* | 0.478 |
| *Checking locks, stove, appliances, etc.* | 0.293 |
| **Factor 4: Sexual an religious obsessions and compulsions** | |
| Forbidden or perverse sexual thoughts/images/impulses | 1.000 |
| Content (of obsession) involves children or incest[a] | 0.842 |
| Content (of obsession) involves homosexuality[a] | 0.841 |
| (Obsession with) sexual behavior toward others (aggressive)[a] | 0.763 |
| Excessive concern with right/wrong, morality | 0.747 |
| Concerned with sacrilege and blasphemy | 0.691 |
| *Violent or horrific images* | 0.323 |
| Factor 5: Hoarding obsessions and compulsions | |
| Hoarding compulsions | 1.000 |
| Hoarding obsessions | 0.931 |
| **Factor 6: Somatic obsessions and checking** | |
| Concern with illness or disease | 1.000 |
| Checking tied to somatic obsessions | 0.711 |
| Excessive concern with a body part or an aspect of appearance (e.g., dysmorphophobia) | 0.620 |
| *Concerned that I will get ill because of contaminant* | 0.430 |
| *Checking that I did not/will not harm self* | 0.466 |
| Concern with illness or disease | 1.000 |

* Items shown in italic show high loadings on more than one factor.

[a] Item missing in the extended 80-item self report version.

Table 7. Kruskal-Wallis and Mann-Whitney *U* Tests comparing scores per item for the different factors between patients with different *BDNF Val66Met* Genotype.*

| | *Val66Val* | *Val66Met* | *Met66Met* | *p*-value | *Val66Met+ Met66Met* | *p*-value | *Val66Val+ Val66Met* | *p*-value | *Met66* | *Val66* | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All patients | | | | | | | | | | | |
| n | 246 | 125 | 21 | | | | | | | | |
| Mean factor 1 items | 0.231±0.249 | 0.187±0.227 | 0.245±0.236 | 0.190 | 0.195±0.229 | 0.178 | 0.216±0.242 | 0.494 | 0.201±0.229 | 0.222±0.245 | 0.400 |
| Mean factor 2 items | 0.237±0.218 | 0.219±0.186 | 0.197±0.186 | 0.801 | 0.216±0.185 | 0.736 | 0.231±0.208 | 0.551 | 0.213±0.185 | 0.233±0.212 | 0.603 |
| Mean factor 3 items | 0.423±0.292 | 0.440±0.300 | 0.367±0.200 | 0.530 | 0.430±0.288 | 0.703 | 0.429±0.294 | 0.388 | 0.422±0.279 | 0.426±0.293 | 0.981 |
| Mean factor 4 items | 0.143±0.205 | 0.112±0.187 | 0.082±0.178 | 0.057 | 0.107±0.185 | **0.046** | 0.133±0.200 | 0.086 | 0.104±0.184 | 0.137±0.202 | **0.017** |
| Mean factor 5 items | 0.199±0.374 | 0.188±0.363 | 0.143±0.359 | 0.728 | 0.182±0.361 | 0.651 | 0.195±0.370 | 0.393 | 0.177±0.360 | 0.197±0.371 | 0.507 |
| Mean factor 6 items | 0.187±0.251 | 0.147±0.228 | 0.152±0.178 | 0.299 | 0.147±0.221 | 0.190 | 0.173±0.244 | 0.814 | 0.148±0.216 | 0.179±0.247 | 0.311 |
| Males | | | | | | | | | | | |
| n | 119 | 59 | 9 | | | | | | | | |
| Mean factor 1 items | 0.204±0.246 | 0.182±0.220 | 0.248±0.210 | 0.536 | 0.190±0.218 | 0.832 | 0.197±0.237 | 0.324 | 0.197±0.217 | 0.200±0.240 | 0.852 |
| Mean factor 2 items | 0.245±0.238 | 0.228±0.203 | 0.133±0.129 | 0.452 | 0.216±0.197 | 0.759 | 0.240±0.226 | 0.215 | 0.206±0.191 | 0.242±0.230 | 0.466 |
| Mean factor 3 items | 0.403±0.300 | 0.441±0.304 | 0.397±0.234 | 0.755 | 0.435±0.295 | 0.486 | 0.416±0.301 | 0.843 | 0.430±0.287 | 0.411±0.300 | 0.644 |
| Mean factor 4 items | 0.171±0.226 | 0.140±0.209 | 0.143±0.247 | 0.546 | 0.140±0.212 | 0.310 | 0.161±0.220 | 0.476 | 0.141±0.215 | 0.165±0.222 | 0.249 |
| Mean factor 5 items | 0.168±0.346 | 0.212±0.385 | 0.222±0.441 | 0.792 | 0.213±0.389 | 0.507 | 0.183±0.359 | 0.867 | 0.214±0.393 | 0.177±0.353 | 0.538 |
| Mean factor 6 items | 0.200±0.269 | 0.168±0.251 | 0.111±0.145 | 0.734 | 0.161±0.240 | 0.444 | 0.189±0.263 | 0.669 | 0.155±0.230 | 0.194±0.265 | 0.417 |
| Females | | | | | | | | | | | |
| | 127 | 66 | 12 | | | | | | | | |
| Mean factor 1 items | 0.255±0.250 | 0.191±0.235 | 0.242±0.263 | 0.171 | 0.199±0.238 | 0.081 | 0.525±0.764 | 0.963 | 0.205±0.241 | 0.242±0.248 | 0.127 |
| Mean factor 2 items | 0.229±0.199 | 0.210±0.171 | 0.244±0.212 | 0.894 | 0.216±0.176 | 0.915 | 0.222±0.190 | 0.705 | 0.219±0.180 | 0.225±0.193 | 0.942 |
| Mean factor 3 items | 0.441±0.285 | 0.439±0.298 | 0.345±0.177 | 0.497 | 0.425±0.284 | 0.892 | 0.440±0.288 | 0.251 | 0.414±0.273 | 0.441±0.286 | 0.560 |
| Mean factor 4 items | 0.117±0.181 | 0.087±0.162 | 0.036±0.089 | 0.111 | 0.079±0.154 | 0.060 | 0.107±0.175 | 0.127 | 0.073±0.147 | 0.111±0.177 | **0.034** |
| Mean factor 5 items | 0.228±0.397 | 0.167±0.343 | 0.083±0.289 | 0.295 | 0.154±0.335 | 0.221 | 0.207±0.380 | 0.192 | 0.144±0.329 | 0.216±0.386 | 0.109 |
| Mean factor 6 items | 0.175±0.232 | 0.127±0.206 | 0.183±0.199 | 0.271 | 0.136±0.205 | 0.294 | 0.159±0.224 | 0.428 | 0.142±0.204 | 0.165±0.227 | 0.539 |
| Mean factor 1 items | 0.255±0.250 | 0.191±0.235 | 0.242±0.263 | 0.171 | 0.199±0.238 | 0.081 | 0.525±0.764 | 0.963 | 0.205±0.241 | 0.242±0.248 | 0.127 |

* *P* values <0.0025 were considered significant; *P* values below the nominal significance level of 0.05 are indicated in bold.

Table 8. Kruskal-Wallis, Mann-Whitney *U* and Fisher's Exact tests comparing YBOCSS scores, age of OC symptom onset and family history between patients with different *BDNF Val66Met* genotype*

| | Val66Val | n | Val66Met | n | Met66Met | n | p | Val66Met+ Met66Met | p | Val66Val+ Val66Met | p | Val66 | Met 66 | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| YBOCS severity score | | | | | | | | | | | | | | |
| All patients | 20.3±7.8 | 248 | 19.5±7.7 | 130 | 20.3±9.1 | 22 | 0.667 | 19.6±7.9 | 0.453 | 20.0±7.8 | 0.885 | 20.1±7.8 | 19.7±8.0 | 0.550 |
| Females | 21.4±7.8 | 130 | 18.7±7.9 | 70 | 20.0±9.6 | 12 | 0.051 | 18.9±8.1 | **0.023** | 20.5±7.9 | 0.991 | 20.8±7.8 | 19.1±8.3 | 0.063 |
| Males | 19.0±7.7 | 118 | 20.5±7.4 | 60 | 20.6±9 | 10 | 0.450 | 20.5±7.6 | 0.217 | 19.5±7.6 | 0.805 | 19.3±7.6 | 20.5±7.7 | 0.239 |
| Age of onset | | | | | | | | | | | | | | |
| All patients | 17.2±10.1 | 235 | 15.9±9.6 | 126 | 21.7±13.8 | 19 | 0.135 | 16.6±10.4 | 0.415 | 16.7±9.9 | 0.127 | 16.9±10.0 | 17.2±10.9 | 0.937 |
| Females | 17.1±10.6 | 120 | 15.9±9.7 | 68 | 26.9±12.2 | 11 | **0.008** | 17.4±10.7 | 0.843 | 16.7±10.3 | **0.002*** | 16.9±10.4 | 18.6±11.2 | 0.192 |
| Males | 17.2±9.6 | 115 | 15.8±9.6 | 58 | 14.5±13.2 | 8 | 0.296 | 15.7±10 | 0.167 | 16.7±9.6 | 0.215 | 16.9±9.6 | 15.5±10.3 | 0.104 |
| Positive family history of OCD | | | | | | | | | | | | | | |
| All patients | 31 | 53 | 15 | 28 | 2 | 6 | 0.577 | 17 | 0.510 | 46 | 0.401 | 92 | 19 | 0.282 |
| Females | 22 | 31 | 9 | 16 | 0 | 4 | **0.022** | 9 | 0.083 | 15 | **0.019** | 53 | 9 | **0.009** |
| Males | 9 | 22 | 6 | 12 | 2 | 2 | 0.310 | 8 | 0.495 | 31 | 0.216 | 24 | 10 | 0.256 |

* *P* values <0.0025 were considered significant; *P* values reaching statistical significance are indicated with an asterisk; *P* values below the nominal significance level of 0.05 are indicated in bold.

**Discussion**

In this study, the *BDNF Val66Met* polymorphism was investigated in a large group of patients with OCD. Female patients with the *BDNF Met66Met* genotype had a later age of onset of OC symptoms and there was a trend for a lower OCD severity in female patients with one or more *BDNF Met66* alleles. Moreover, female patients with a negative family history of OC symptoms more often had the *BDNF Met66Met* genotype. Although these results should be considered preliminary, given the low number of female patients with the *BDNF Met66Met* genotype (n=12) and that data on age of onset of OC symptoms were collected retrospectively, the findings suggest that the *BDNF Met66* alleles may have a protective role in females with OCD. The finding of a protective effect of the *BDNF 66Met* allele is in line with a previous study (including patients of both sexes) that found the *BDNF 66Met* alleles to be *under*-transmitted in early onset OCD cases (Hall et al., 2003). Early onset seems to be linked to more severe OCD (Fontenelle et al., 2003; Sobin et al., 2000). Other studies in which no association was found between the *BDNF Val66Met* variant and age of onset had the drawback that smaller samples were used which might have hampered power to detect between-group differences (Wendland et al., 2007; Zai et al., 2005).

Little is known about a sex-specific association between the *BDNF Val66Met* polymorphism, OCD symptom severity and age of onset of OC symptoms. To our knowledge, the only other study that has directly investigated this, found an association between the *BDNF Met66* allele and an earlier age of onset of OCD in males (Hemmings et al., 2008). In apparent contrast, our findings suggested a significant association between the *BDNF Met66Met* genotype in females and later age of OC symptom onset, coupled with a trend towards lower OC symptom severity. Our findings may be analogous to animal studies that have suggested sex-dependent differences in heterozygous $BDNF^{+/-}$ knockout mice, with female heterozygous *BDNF*/homozygous serotonin transporter gene (*5HTT*)-knockout mice showing less increase in anxiety-like behaviors as well as less reduction of serotonin concentrations in several brain regions than males (Ren-Patterson et al., 2006). These findings suggest that female sex may protect against reduced *BDNF* function in mice, at least in the presence of *5HTT*-deficiency. The relationship between sex and serotonin levels might result from differences in hormone status between men and women. In estrogen-receptor β-knockout mice decreased concentrations of serotonin and dopamine have been found, combined with increased anxiety reactions, independent of current estradiol suppletion (Imwalle et al., 2005). Moreover, it has been shown that estrogen increased expression of tryptophan hydroxylase 2 (TPH2), the rate limiting enzyme in

serotonin synthesis, in the caudal region of the raphe nuclei of ovariectomized rats and that expression of TPH2 mRNA in this region is correlated with anxiety-like behavior (Hiroi et al., 2006). Therefore, sex dependent effects on anxiety symptoms might be the result of a serotonin enhancing effect of estrogen at the level of the caudal raphe nuclei. Recently, increasing evidence suggest that changes in the glutamatergic system may be implicated in OCD (Pittenger et al., 2006). BDNF has been shown to influence the glutamatergic system by alteration of the composition and plasticity of glutamatergic synapses (Carvalho et al., 2008). Therefore, the effects of the *BDNF Val66Met* polymorphism may also be mediated by the glutamatergic pathway.

There are several lines of evidence in support of our findings indicating that age of onset of OC symptoms and OCD symptom severity are important aspects of the OCD phenotype that may have specific genotypic underpinnings. Previous studies suggest that age of onset in OCD has two distributions (Delorme et al., 2005). Moreover, early onset OCD has been found to represent a more familial phenotype of the disorder (Delorme et al., 2005; Lenane et al., 1990; Pauls et al., 1995).

Item-level factor analysis of the YBOCS-CL identified six OCD symptom dimensions. The contamination/cleaning, symmetry/ordering, and hoarding symptom dimensions were similar to the factors consistently found in previous category-based factor analyses. The aggressive obsession/checking factor often found in category-based factor analysis usually comprises aggressive, sexual, and religious obsessions and in some studies this factor also included somatic obsessions. In our item-level factor analysis, these symptoms seemed to be divided over three factors: aggressive obsessions/checking, sexual and religious obsessions and somatic obsessions and related checking.

We found a trend for higher mean score per item for Factor 4 (sexual and religious obsessions) in patients with the *BDNF Val66Val* genotype than in patients with other genotypes. This may suggest a protective effect for the *BDNF Met66* alleles against sexual and religious obsessions. This finding has to be considered with caution however, since a considerable amount of missing data were imputed for items in this factor. Two previous studies found that the *BDNF Val66Met* polymorphism was not significantly associated with any OCD symptom dimension (Alonso et al., 2008a; Wendland et al., 2007). However, these studies comprised smaller sample sizes, category-based analyses and only one study specifically investigated the sexual and religious obsession symptom category (Alonso et al., 2008a).

A limitation of our study is the limited power to detect recessive effects of the *BDNF Met66* allele. Since the allele frequency of the *BDNF Met66* allele is low, larger sample sizes are needed to detect recessive effects. Only the *Val66Met* polymorphism was

investigated, which is a limitation of this study. To our knowledge, the *Val66Met* polymorphism is the only known functional polymorphism in the *BDNF* gene. Therefore, we thought it was justified to focus on this polymorphism only. A limitation of case-control association studies is that these can be biased by population stratification (Thomas and Witte, 2002). Family-based studies have been developed to circumvent this problem (Schulze and McMahon, 2002). Alternatively, multiple markers can be typed to test and correct for possible population stratification by genomic control or structured association methods (Pritchard and Donnelly, 2001). The use of these methods in future studies would be desirable.

Further, gene-gene interactions that might confer increased risk for OCD or dimensional OCD phenotypes were not investigated.

To our knowledge, only two studies investigated gene-gene interaction involving the *BDNF* gene both with negative results. Both studies have a relatively limited sample size, which may have caused their negative results. The first study investigating interaction between the *BDNF Val66Met* polymorphism and the *5HTT* gene promoter polymorphism found no evidence for a gene-gene interaction in OCD (Wendland et al., 2007). However, the negative result of this study might have been the consequence of not taking into account the effect of negative life events on the interaction between the *BDNF Val66Met* and the *5HTTPR* polymorphism, as found in studies in depression (Kaufman et al., 2006; Kim et al., 2007). The second study investigated the interaction between the *BDNF* gene and the gene encoding its specific receptor, the neurotrophic tyrosine kinase 2 gene (*NTRK2*) gene (Alonso et al., 2008a). In summary, studies investigating the role of gene-gene interaction of the *BDNF Val66Met* polymorphism and other genes as well as gene x environment interactions in the etiology of OCD are warranted.

In conclusion, our results suggest that in females, the *BDNF Met66Met* genotype may be associated with a late-onset form of OCD and the *BDNF Val66Val* genotype with a more severe form of OCD. Future studies including relevant variables such as sex, age of onset, severity of OC symptoms and OC symptom dimensions obtained by item level factor analysis in a sample large enough to detect recessive effects of the *BDNF Val66Met* polymorphism are needed to replicate and extend our current findings.

Chapter 5

# The Role of the COMT Val$^{158}$Met Polymorphism in the Phenotypic Expression of Obsessive-Compulsive Disorder

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**Abstract**

*Obsessive-Compulsive Disorder (OCD) is characterized by the presence of obsessions and compulsions, and shows considerable phenotypic variability. Family and twin studies have indicated a genetic component in the etiology of OCD, and the catechol-O-methyl transferase (COMT) gene is an important candidate gene for OCD. This study investigates the influence of the functional COMT Val158Met polymorphism on the phenotypic expression of OCD, using an item-level factor-analytic approach in a large sample. The COMT Val158Met variant was genotyped in 373 patients and 462 controls. It was tested whether there was an association between the COMT Val158Met polymorphism and OCD or dimensional phenotypes such as YBOCS severity score, age of onset of obsessive-compulsive symptoms and six symptom dimensions recently found in a large item-level factor-analytic study (Katerberg et al., submitted). We further investigated possible sex-specific associations between the COMT Val158Met polymorphism and OCD or dimensional phenotypes. There was a trend for an association of the COMT 158Met allele with OCD in males, and an interaction between the COMT Val158Met genotype and sex on the somatic and sensory phenomena symptom dimension, with females showing lower scores. In conclusion, a dimensional approach seems fruitful in detecting genes of importance for OCD.*

**Introduction**

Obsessive-Compulsive Disorder (OCD) is characterized by repetitive intrusive recurrent thoughts (obsessions) and/or repetitive behaviors (compulsions) (American Psychiatric Association, 1994). Family and twin studies have indicated a genetic component in the etiology of at least some forms of OCD (Hettema et al., 2001; van Grootheest et al., 2005).

However, there has been only limited progress in the search for genetic susceptibility factors for OCD. The three published whole genome scans for OCD do not show regions with consistently high LOD-scores (Hanna et al., 2002, 2007; Shugart et al., 2006). Association studies investigating several candidate genes in OCD have been performed, mostly with inconsistent results (Hemmings and Stein, 2006).

Several lines of evidence suggest an increased dopaminergic neurotransmission in the midbrain in OCD (Denys et al., 2004c). Cathecholamine-*O*-methyl transferase (COMT) is involved in the degradation of dopamine and norepinephrin, and is of particular importance for the clearance of dopamine in the prefrontal cortex since DAT (dopamine transporter) levels are very low in this brain region. The *COMT* gene is located at chromosome 22q11 and contains a functional polymorphism that causes an amino acid substitution at position 158 (*Val158Met*). The *Met158Met* genotype is associated with a 3-4 times reduced enzyme activity compared to the *Val158Val* genotype, and the *Val158Met* genotype has intermediate activity (Lachman et al., 1996a). Therefore, the *COMT Val158Met* polymorphism is among the most widely studied polymorphisms in OCD. In eight studies a case-control design was used to investigate the association between the *COMT Val158Met* polymorphism and OCD. In four of these studies the *COMT 158Met* allele was associated with OCD in males (Denys et al., 2006a; Karayiorgou et al., 1997; Pooley et al., 2007; Poyurovsky et al., 2005). In one study, the *COMT Val158Met* genotype was associated with OCD (Niehaus et al., 2001). Three studies showed no association between any of the *COMT Val158Met* polymorphism genotypes or allele frequencies between cases and controls (Erdal et al., 2003; Meira-Lima et al., 2004; Ohara et al., 1998b). Four studies used a family-based approach. One of these studies found an association of the *COMT 158Met* allele in males, one found an association between the *COMT 158Met* allele and OCD in females, the third study found an association of homozygosity for the *COMT Val158Met* polymorphism and OCD, whereas the fourth study found no preferential transmission of any of the alleles (Alsobrook et al., 2002b; Karayiorgou et al., 1999; Schindler et al., 2000; Walitza et al., 2008). Two meta-analyses of studies investigating the *COMT Val158Met* polymorphism in OCD have been performed (Azzam and Mathews, 2003; Pooley et al., 2007). The first study analyzed case-control studies and family-based studies separately and found

insufficient evidence to support an association between the *COMT Val158Met* polymorphism and OCD. The second meta-analysis included case-control studies only and found evidence for an association between the *COMT Val158Met* polymorphism and OCD in males.

One of the causes of these inconsistent results may be the heterogeneity of OCD. A strategy to reduce this problem of heterogeneity is to define more homogeneous phenotypes based on clinically defined characteristics. The Yale-Brown Obsessive-Compulsive Scale (YBOCS) is designed to assess the severity of OC symptoms and is accompanied by the YBOCS symptom checklist (YBOCS-CL), designed to screen for the presence of a number of frequently encountered symptoms (obsessions and compulsions) in OCD (Goodman et al., 1989a, 1989b). Factor analysis on the YBOCS-CL symptom categories is a frequently used method to define more homogeneous symptom dimensions of OCD. Generally, four or five factors are consistently found (Mataix-Cols et al., 2005). Family and segregation studies suggest that they are familial and that there is evidence of differential genetic transmission (Alsobrook et al., 1999; Hasler et al., 2007; Leckman et al., 1997). This has encouraged several researchers to use these symptom dimensions in molecular genetic studies of OCD.

The first aim of the study was to investigate whether the frequently found symptom dimensions were also present in our sample of patients with OCD. The second aim of the study was to investigate whether the *COMT Val158Met* polymorphism is associated with one or more of these symptom dimensions or other characteristics such as age of onset of obsessive-compulsive (OC) symptoms and YBOCS severity scores. Since sex differences have been described for both clinical and genetic findings in OCD (Labad et al., 2008; Lochner et al., 2004), an additional aim was to investigate sex-specific associations of the *COMT Val158Met* genotype with clinical characteristics of OCD such as OC symptom dimensions, age of onset of OC symptoms, YBOCS severity score, family history of OC behavior, and tic-relatedness.

**Recruitment**

Patients were recruited at three sites in the Netherlands (the University Medical Center Utrecht, the University Medical Center in Groningen, and the VU Medical Center in Amsterdam).

In Utrecht patients were recruited among patients diagnosed with primary OCD at the anxiety research unit of the University Medical Center. Most of these patients were enrolled in a previous factor analytic study and/or a previous association study (Denys et al., 2004b, 2006a). Patients recruited in Amsterdam were from the psychiatric outpatient clinic of the VU Medical Center, referrals from other mental health care institutions and

meetings of the Dutch Tourette Association. In Groningen patients were recruited from the (outpatient) clinic of the University Medical Center, referrals from other mental health care institutions, patient societies and advertisements in local newspapers.

Detailed information on the ethnicity of the participants was not collected. However, based on physical appearance and surname >95% of the patients were Caucasian. The control subjects consisted of 312 controls recruited from the general Dutch population and 150 Caucasian controls from a previous case-control study (Denys et al., 2006a). The study was approved by the medical ethical review boards of the participating centers.

*Clinical Evaluation*

Patients were diagnosed with current (Utrecht) or lifetime (Groningen and Amsterdam) OCD according to DSM-IV criteria. The Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998) (Utrecht and Groningen) or SCID-I (First et al., 1998) (Amsterdam) were used to confirm the diagnosis. Tourette's disorder and other tic disorders were diagnosed according to DSM-IV criteria. Data on severity of obsessive compulsive symptoms at assessment were collected using the YBOCS severity scale (YBOCS-SS) (Goodman et al., 1989a, 1989b). Data on Obsessive Compulsive symptoms were collected using the YBOCS-CL (Goodman et al., 1989a, 1989b). For 110 patients in Amsterdam, an extended 80-item self-report version of the YBOCS-CL was used, for the other patients a 74-item clinician rated version of the YBOCS-CL was used. This 80-item self-report YBOCS-CL was translated from the version used in the TSA genetics consortium on Tourette's disorder and within the scope of the OCF international collaboration on the genetics of OCD. Lifetime presence of the symptoms was coded. Items were coded as 1 when the symptom was present currently or in the past and as 0 when the symptom had never been present. To measure symptom severity, we used the original interview-based YBOCS severity scale in all patients. In addition, data on age of onset of Obsessive Compulsive symptoms and family history of OC symptoms were collected. Family history was considered to be positive if the subject reported on the presence of recognizable and bothering OC symptomatology in at least one first-degree family member.

*Genotyping*

DNA from patients was collected using blood, buccal swabs and, in a few cases, saliva. DNA from controls was collected from blood or buccal swabs.

DNA was isolated from blood using a chloroform/isopropanol extraction (Meulenbelt et al., 1995), from buccal swabs using a salting out procedure (Miller et al., 1988) of from sputum using an Oragene DNA self collection kit (DNA Genotek Inc., Ottawa, Canada) according to manufacturers instructions. One sample was amplified using a

GenomiPhi™ DNA amplification kit (GE Healthcare Europe GmbH) according to manufacturer's instructions. A total of 373 patients (151 males, 222 females, mean age 37±12; range 9-78) were successfully genotyped.

Patients recruited in Utrecht (n=152) were genotyped in an association study as described previously (Denys et al., 2006a). Briefly, a 96-bp fragment comprising the *COMT Val158Met* polymorphism was amplified by polymerase chain reactions (PCR). PCR products were digested with *Nla*III restriction enzyme. Digestion with *Nla*III yields two fragments for the *Val* allele and three fragments for the *Met* allele. Fragments were resolved on a 2.5% agarose gel and visualized by ethidium bromide staining.

Patients recruited in Amsterdam and Groningen (n=221) were genotyped using a Taqman® Drug metabolism genotyping assay (assay number C__25746809_50) according to manufacturer's instructions using a 5 or 2 µl final reaction volume).

*Statistical Analysis*

Genotype and allele frequencies in patients and controls were compared using $\chi 2$ tests. Power calculations were performed with the genetic power calculator (Purcell et al., 2003) assuming a disease prevalence of 2% (Angst et al., 2004; Weissman et al., 1994). Hardy-Weinberg equilibrium was tested using $\chi 2$ tests with Microsoft Office Excel.

Item-level factor analysis was performed on the individual YBOCS-CL items. In total, 58 of the 74 items of YBOCS-CL were included in the analysis. Ten symptoms encoded other symptoms were excluded from the analysis because these symptoms were considered to be too ambiguous in their interpretation. Four additional items were excluded because of high rates of missing values (symmetry obsessions with magical thinking, symmetry obsessions without magical thinking, contamination obsessions with no concern than how it might feel, and checking of stoves etc.) and two symptoms were excluded because of low endorsement (trichotillomania and other self damaging behaviors).

Factor analysis was performed using SAS (v9.1.3) and Mplus (v4.2) in which the factor analysis estimation was based on weighted least-squares estimates using a diagonal weight matrix. Tetrachoric correlation coefficient estimates were used for the item-level correlation matrix (via the %polychor macro in SAS). Full information maximum likelihood used by MPlus as a default was used to handle missing data. All data were used in the analyses (i.e., no patients were excluded from the analysis due to missing data). The mean score per item for each of the obtained factors was calculated for each patient. This score represents the prominence of this symptom dimension for each patient.

**Genotype-Phenotype Correlations**

Genotype-phenotype correlations were investigated using univariate ANOVA both for main effects of *COMT Val158Met* genotype and sex and for an interaction effect between *COMT Val158Met* genotype and sex. Empirical p-values for these tests were calculated based on 1000 random permutations using R statistical package in order to obtain robust p-values (R Development Core Team, 2008). Differences in categorical variables between patients with different *COMT Val158Met* genotypes were compared using $x^2$ tests. To investigate probable heterosis effects, patients with the heterozygous *COMT Val158Met* genotype were compared to patients with homozygous *COMT Val158Met* genotypes. In addition, allelic association was investigated. Missing values in the YBOCS-SS were imputed using Solas™ 3.2 (Statistical Solutions, Ltd; Cork, Ireland) using predictive model based imputation.

All tests were performed using SPSS version 14.0 unless otherwise stated. Eleven phenotypes were tested (OCD as a dichotomous trait, age of onset of OC symptoms, YBOCS severity score, family history, the presence of tics and 6 symptom dimensions). These phenotypes were tested for the entire sample and for males and females separately. To control for multiple testing, 11×2 tests were considered, and a p-value of 0.05/22=0.0023 was considered significant for these tests.

**Sample Size and Power Considerations**

Larger sample sizes tend to produce more stable variable loadings across repeated sampling and more precise estimates of population loadings (MacCallum et al., 1999). Therefore, we also included non-genotyped participants in the factor analysis. The total number of patients included in the item-level FA was 1218, were the result of a collaboration within the Obsessive-Compulsive Foundation genetic consortium (Katerberg et al., 2009b).

Patients were collected at five different sites: San Francisco (n=124), Boston (n=329), Amsterdam (n=229), Utrecht (n=387), and Groningen (n=149). The group contained 54.5% females, mean age at assessment±SD was 35.0±11.9 years; mean age of onset±SD was 16.9±9.3 years, and mean YBOCS severity score±SD was 24.4±8.2.

In the total sample, based on the allele frequency in our control population, the power to detect a relative risk of 2 for OCD in the *COMT Val158Met* and *Met158Met* genotypes compared to the *Val158Val* genotype was 80.8, 54.9, and 46.2% in the total sample, males and females, respectively. The power to detect a relative risk of 2 in the *COMT Met158Met* genotype compared to the *Val158Met* and *Val158Val* genotypes was 98.5%,

in the total sample, 73.9% in males and 89.4% in females. The power to detect an effect of the *COMT 158Met* allele that accounts for 2.5% of the variance of a quantitative trait, assuming that the *COMT 158Met* allele is the risk allele based on the allele frequencies of the patients included in the factor analysis is 81.2, 45.0, and 58.6% for an additive effect in the total sample, males and females respectively and 72.3, 35.7, and 48.1% for a dominant effect in the total sample, males and females, respectively.

## Results

Demographic and clinical characteristics of the genotyped sample are summarized in table 1. Mean age at assessment was 37.1, mean age at onset was 17.2 and mean YBOCS severity score was 21.7.

Table 1. Demographics and clinical characteristics of the OCD patients in the different cohorts.

| | Total cohort | Utrecht | Amsterdam | Groningen | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| n | 373 | 152 | 147 | 74 | | |
| Age (mean±SD) | 37.1±11.6 (n=358) | 36.4±11.4 (n=137) | 36.1±11.0 | 40.5±12.7 | 5.633 | 0.060 |
| Female (%) | 222 (59.5) | 97 (63.8) | 80 (54.4) | 45 (60.8) | 2.801 | 0.246 |
| Age of onset (mean±SD) | 17.2±9.1 (n=328) | 17.7±8.3 (n=135) | 15.3±8.0 (n=123) | 19.7±11.4 (n=70) | 8.341 | 0.015 |
| YBOCS severity (mean±SD) | 21.7±7.7 (n=330) | 24.9±5.7 (n=123) | 21.0±8.2 (n=133) | 17.9±7.6 (n=74) | 38.846 | <0.001 |
| Positive family history (%) | 103 (37.6) (n=274) | 41 (27.2) (n=151) | 62 (50.4) (n=123) | n.a. | 15.624 | <0.001 |
| Tics (n present) | n.a. | n.a. | 65 (46.6) (n=140) | n.a. | | |

## Case-Control Study

Genotypes for the *COMT Val158Met* polymorphism were available for 373 patients with OCD (151 males, 222 females) and 462 control subjects (235 males, 227 females). Genotype and allele frequencies for the *COMT Val158Met* variant are summarized in table 2. Genotype distribution was in Hardy-Weinberg equilibrium for both patients and controls, also after stratification by sex. There was no statistically significant difference in genotype (p=0.901) or allele frequency (p=0.733) between controls from the different sites. There was no statistically significant difference in genotype (p=0.977) or allele (p=0.829) frequency between male controls from the different sites, nor were there statistically significant differences in genotype (p=0.971) or allele (p=0.990) frequency between female controls from the different sites.

There was no difference in genotype (p=0.516) or allele (p=0.672) frequency between OCD patients from different sites. Further, there were no differences in genotype (p=0.196) or allele (p=0.339) frequency between male OCD patients from different sites, nor were there differences in genotype (p=0.711) or allele (p=0.370) frequency between female patients from the different sites. Therefore, the cases and controls from the different sites were pooled to increase sample size for the subsequent analyses.

There was a trend for an association of the *COMT Val158Met* allele with OCD in males (p=0.039); not significant after correction for multiple testing).Interestingly, there was an association between sex and genotype (p=0.044) and allele frequency (p=0.012) in the control sample. Females had an increased frequency of the *COMT 158Met* allele and *COMT Met158Met* genotype (p=0.035) compared to males.

Table 2. Case-control association study ($\chi^2$ tests).

| | COMT Met/Met | COMT Val/Met | COMT Val/Val | $\chi^2$ | p | COMT Met | COMT Val | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|---|---|
| OCD | 108 | 183 | 82 | | | 399 | 347 | | |
| | (29.0%) | (49.1%) | (22.0%) | | | (53.5%) | (46.5%) | | |
| Controls | 122 | 228 | 112 | | | 472 | 452 | | |
| | (26.4%) | (49.4%) | (24.2%) | 0.943 | 0.624 | (51.1%) | (48.9%) | 0.955 | 0.328 |
| Male OCD | 45 (29.8%) | 75 | 31 | | | 165 | 137 | | |
| | | (49.7%) | (20.5%) | | | (54.6%) | (45.4%) | | |
| Male controls | 52 (22.1%) | 117 | 66 | | | 221 | 249 | | |
| | | (49.8%) | (28.1%) | 4.243 | 0.120 | (47.0%) | (53.0%) | 4.264 | **0.039** |
| Female OCD | 63 (28.4%) | 108 | 51 | | | 234 | 210 | | |
| | | (48.6%) | (23.0%) | | | (52.7%) | (47.3%) | | |
| Female controls | 70 (30.8%) | 111 | 46 | | | 251 | 203 | | |
| | | (48.9%) | (20.3%) | 0.612 | 0.737 | (55.3%) | (44.7%) | 0.603 | 0.437 |

**Factor analysis**

In the item-level factor analysis (Table 3), a six factor model was found, explaining 66% of the variance in the unrotated solution. The following factors were found: Factor 1: Taboo-Sexual, aggressive, and religious obsessions; Factor 2: Contamination obsessions, and cleaning compulsions; Factor 3: Rituals and superstition-e.g., repeating, superstition, and mental rituals; Factor 4: Fear of harm-e.g., fear to cause harm to others and excess morality; Factor 5: Intolerance of uncertainty, characterized by hoarding, ordering, arranging, and fear of losing things; and Factor 6: Somatic and sensory phenomena. Factors and factor loadings are shown in table 3.

**Genotype-Phenotype Correlations**

There was an interaction between the *COMT Val158Met* genotype and sex on mean item scores for factor 6 (somatic and sensory phenomena) at a trend level (p=0.024), women with the *COMT Val158Met* genotype showing lower scores on this factor (table 4). Table 5 shows the results of comparisons of a positive family history of OC behavior and the presence of comorbid tics between patients with different genotypes. There were no differences in genotype or allele frequency between patients with and patients without a positive family history of OC behavior, or between patients with versus without comorbid tics.

Table 3. Factors and factor loadings found by item-level factor analysis (items with loadings <0.395 are omitted).

| **Factor 1: Taboo** | |
|---|---|
| Sexual behavior toward others (aggressive) | 0.832 |
| Forbidden/perverse thoughts, images, impulses | 0.813 |
| Sexual obsessions; content involves children or incest | 0.785 |
| Fear will act on unwanted impulses | 0.766 |
| Fear of blurting obscenities/insults | 0.746 |
| Sexual obsessions; content involves homosexuality | 0.727 |
| Violent or horrific images | 0.717 |
| *Fear might harm others* | 0.692 |
| Fear might harm self | 0.688 |
| Fear of doing something embarrassing | 0.653 |
| Fear will steal things | 0.653 |
| Excessive concern with sacrilege and blasphemy | 0.478 |
| *Intrusive (nonviolent) images* | 0.452 |
| *Fear of saying certain things* | 0.441 |
| *Checking that did not/will not harm self* | 0.434 |
| *Intrusive nonsense sounds, words or music* | 0.431 |
| *Fear will harm others because not careful enough* | 0.405 |

| **Factor 2: Contamination/cleaning** | |
|---|---|
| Excessive or ritualized hand washing | 0.874 |
| Concerns with dirt or germs | 0.869 |
| Other measures to prevent/remove contaminant contact | 0.823 |
| Excessive or ritualized showering, grooming etc. | 0.769 |
| Cleaning of household items/inanimate objects | 0.761 |
| Concerns with bodily waste or secretions | 0.755 |
| Bothered by sticky substances or residues | 0.729 |
| Excessive concerns with animals | 0.718 |
| Excessive concern with household items | 0.701 |
| Excessive concerns with environmental contaminants | 0.658 |
| Concerned will get ill because of contaminant | 0.638 |
| *Concerned will get others ill from contaminant* | 0.552 |
| **Factor 3: Rituals and superstition** | |
| Need to repeat routine activities | 0.768 |
| Lucky/unlucky numbers | 0.738 |
| Need to touch, tap or rub | 0.707 |
| Superstitious behaviors | 0.651 |
| Counting compulsions | 0.641 |
| Superstitious fears | 0.586 |
| Colors with special significance | 0.559 |
| Ritualized eating behaviors | 0.508 |
| *Mental rituals* | 0.499 |
| *Re-reading or re-writing* | 0.496 |
| *Ordering/arranging compulsions* | 0.428 |
| *Rituals involving blinking or staring* | 0.418 |
| *Fear of saying certain things* | 0.400 |

*Items shown in italic show high loadings on more than one factor

Table 3 (continued). Factors and factor loadings found by item-level factor analysis (items with loadings <0.395 are omitted).

| **Factor 4: Fear of harm** | |
|---|---|
| Checking that did not/will not harm others | 0.765 |
| Fear will be responsible for something else terrible happening | 0.730 |
| *Fear will harm others because not careful enough* | 0.695 |
| Checking that nothing terrible did/will happen | 0.595 |
| Checking that did not make mistake | 0.518 |
| *Concerned will get others ill from contaminant* | 0.512 |
| *Fear might harm others* | 0.504 |
| *Checking that did not/will not harm self* | 0.460 |
| Excessive concern with right/wrong, morality | 0.423 |
| *Rituals involving blinking or staring* | 0.415 |
| Measures (no checking) to prevent harm to self, others or terrible consequences | 0.397 |
| Checking that did not/will not harm others | 0.765 |

| **Factor 5: Intolerance of uncertainty** | |
|---|---|
| Hoarding compulsions | 0.665 |
| Excessive listmaking | 0.628 |
| Hoarding obsessions | 0.619 |
| *Ordering/arranging compulsions* | 0.587 |
| Fear losing things | 0.572 |
| *Re-reading or re-writing* | 0.535 |
| Need to know or remember | 0.493 |
| Fear not saying the right thing | 0.476 |
| **Factor 6: Somatic and sensory phenomena** | |
| Checking tied to somatic obsessions | 0.612 |
| *Intrusive nonsense sounds, words or music* | 0.571 |
| *Intrusive (nonviolent) images* | 0.562 |
| Excessive concern with body part or appearance | 0.552 |
| Bothered by certain sounds/noises | 0.525 |
| Concern with illness or disease | 0.521 |
| *Mental rituals* | 0.488 |
| Need to tell, ask or confess | 0.401 |

*Items shown in italic show high loadings on more than one factor

Table 4. Results of associations between *COMT Val158Met* genotypes and alleles and mean item score for the different factors, YBOCS severity and age of onset.

| | n | Adjusted $r^2$ | Sex (p) | COMT genotype (p) | COMT genotype*sex (p) | Adjusted $r^2$ | Sex (p) | COMT Val/Met (p) | COMT Val/Met *sex (p) | Adjusted $r^2$ | Sex (*p*) | COMT allele (p) | COMT allele*sex (p) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taboo | 320 | -0.004 | 0.118 | 0.706 | 0.747 | -0.001 | 0.105 | 0.875 | 0.861 | 0.005 | **0.027** | 0.391 | 0.457 |
| Contamination/cleaning | 320 | 0.001 | 0.080 | 0.696 | 0.567 | 0.005 | 0.086 | 0.435 | 0.315 | 0.006 | **0.016** | 0.720 | 0.635 |
| Rituals and superstition | 320 | 0.002 | 0.798 | 0.329 | 0.170 | -0.004 | 0.838 | 0.648 | 0.228 | 0.001 | 0.724 | 0.139 | 0.258 |
| Fear of harm | 320 | -0.008 | 0.967 | 0.378 | 0.781 | -0.004 | 0.959 | 0.234 | 0.520 | -0.004 | 0.903 | 0.528 | 0.984 |
| Intolerance of uncertainty | 320 | <0.001 | **0.110** | 0.814 | 0.393 | 0.004 | 0.114 | 0.571 | 0.214 | 0.005 | **0.024** | 0.760 | 0.564 |
| Somatic and sensory phenomena | 320 | 0.017 | 0.247 | 0.293 | **0.024** | 0.015 | 0.275 | 0.536 | **0.018** | 0.003 | 0.111 | 0.161 | 0.412 |
| YBOCS severity | 330 | 0.011 | **0.029** | 0.883 | 0.157 | 0.013 | **0.027** | 0.780 | 0.113 | 0.011 | **0.006** | 0.580 | 0.272 |
| Age of onset | 328 | -0.006 | 0.900 | 0.747 | 0.303 | -0.001 | 0.899 | 0.528 | 0.158 | -0.003 | 0.866 | 0.733 | 0.401 |

Adjusted $r^2$: adjusted correlation coefficient squared, COMT genotype: main effect of *COMT* genotype, sex: main effect of sex, *COMT* genotype*sex: effect of interaction between *COMT* genotype and sex, COMT Val/Met: main effect of presence of *COMT Val/Met* genotype, COMT Val/Met*sex: effect of interaction between sex and presence of *COMT Val/Met* genotype, COMT allele: effect *COMT* allele, COMT allele*sex: effect of interaction between *COMT* allele and sex.

Table 5. Difference in family history and presence of tics between patients with different *COMT Val158Met* genotypes and between the *COMT Val158Met* alleles

| | COMT Met/Met | n | COMT Val/Met | n | COMT Val/Val | n | p | COMT Val/Met+ Met/Met | p | COMT Val/Val+ Val/Met | p | COMT Val/Val+ Met/Met | p | COM T Met | COM T Val | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Positive family history | | | | | | | | | | | | | | | | |
| All patients | 28 | 77 | 49 | 131 | 26 | 66 | 0.931 | 77 | 0.729 | 75 | 0.793 | 54 | 0.951 | 101 | 105 | 0.706 |
| Male patients | 10 | 34 | 20 | 50 | 14 | 28 | 0.253 | 30 | 0.180 | 34 | 0.158 | 24 | 0.889 | 40 | 48 | 0.082 |
| Female patients | 18 | 43 | 29 | 81 | 12 | 38 | 0.667 | 47 | 0.478 | 41 | 0.387 | 30 | 0.870 | 65 | 53 | 0.334 |
| Tics | | | | | | | | | | | | | | | | |
| All patients | 17 | 39 | 30 | 67 | 18 | 34 | 0.677 | 47 | 0.382 | 48 | 0.676 | 35 | 0.707 | 64 | 66 | 0.426 |
| Male patients | 10 | 17 | 14 | 31 | 9 | 18 | 0.664 | 24 | 1.000 | 23 | 0.398 | 19 | 0.459 | 34 | 32 | 0.601 |
| Female patients | 7 | 22 | 16 | 36 | 9 | 16 | 0.318 | 23 | 0.236 | 25 | 0.197 | 16 | 0.839 | 30 | 34 | 0.126 |

**Discussion**

We investigated phenotype-genotype correlations for the *COMT Val158Met* polymorphism in OCD patients, using age of onset of obsessive-compulsive symptoms and YBOCS severity score, as well as factor-analyzed OC symptom dimensions. To our knowledge, the sample described in this paper is the largest sample of OCD patients investigated for *COMT Val158Met* polymorphism to date, using an item-level based factor analytic approach.
In the case-control study, we found a trend for an association between OCD and the *COMT 158Met* allele in males, an association that was largely accounted for by the Utrecht subgroup of our sample. This finding is in line with the association between the *COMT158Met* allele and OCD in males in a recent meta-analysis (Pooley et al., 2007). Schindler noted that the sex-specific association could be the result of sampling rather than indicating true sex differences (Schindler et al., 2000). An early age of onset may identify a more familial phenotype of the disorder (Chabane et al., 2005; Delorme et al., 2005). Early onset is therefore suggestive of increased genetic load. Since males demonstrate an earlier age of onset of OCD, the role of genetic factors in the etiology of OCD may in general be higher in males. It is therefore interesting, that in the genotyped group of patients from Utrecht, in which the association between the *COMT 158Met* allele was found, the age of onset for males was significantly lower compared to females, whereas in the pooled sample from Amsterdam and Groningen there was no significant difference in age of onset between males and females. Moreover, the finding of a sexually dimorphic association between OCD and the *Val158Met* polymorphism points to a different role of this specific polymorphism in OCD in males compared to females. This is consistent with the finding of sex differences in COMT activity. It has been shown in post-mortem tissue that females have a lower level of COMT activity in the dorsolateral prefrontal cortex (Chen et al., 2004). This sex difference in COMT activity might be caused by down-regulation of COMT expression and activity by estradiol (Karayiorgou et al., 1999). It has been suggested that estrogen withdrawal may worsen OC symptoms, since women have reported that their symptoms increase prior to menstruation or postpartum or at the beginning of menopause (Labad et al., 2005; Vulink et al., 2006; Williams and Koran, 1997). Therefore, it can be hypothesized that a sex-specific association between OCD and COMT might be explained by a different regulation of COMT expression and activity through differences in the levels of estrogen. In support of this, estrogen deficient male mice develop compulsive behavior such as excessive grooming and barbering (Hill et al., 2007). These behaviors were associated with reduced hypothalamic COMT levels (Hill et al., 2007). No obsessive-compulsive behavior has been reported in *COMT* knockout mice and *COMT*-gene disrupted mice (Gogos et al., 1998; Haasio et al., 2003). As mentioned by Hill et al., this may be

caused by the fact that behavior in these mice was studied at an early age, whereas obsessive-compulsive behavior in estrogen deficient male mice was not expressed until a later age (Hill et al., 2007).

De Mille et al. state that the estrogen responsive region in the P2 promoter is intriguing considering the sex differences in penetrance and expressivity in many neuropsychiatric diseases (DeMille et al., 2002). The differences in allele frequency between males and females in our control sample are remarkable. A previous study investigating the role of the *COMT* gene in schizophrenia found a difference in allele frequency between males and females from the general population for another SNP (rs 165599) in the *COMT* gene (Shifman et al., 2002). Further research to investigate the implications of these findings is warranted.

The previously reported trend between the *COMT Met158Met* genotype and a higher age of onset in males (Karayiorgou et al., 1999) was not replicated in this study.

We did not find any difference in YBOCS-severity scores between patients with different *COMT Val158Met* genotypes. To our knowledge, only one study that investigated the relationship between YBOCS severity score and the *COMT Val158Met* genotype found an association between the high activity genotype and increased YBOCS-severity scores in young males with recent-onset schizophrenia (Zinkstok et al., 2008). We found a trend for lower scores on the somatic obsessions and sensory phenomena factor in females with the *COMT Val158Met* genotype. This means that the intermediate activity *COMT Val158Met* genotype might protect women with this genotype against somatic obsessions. Previous work on symptom dimensions showed an association between the *COMT Met158Met* genotype and the *COMT 158Met* allele and hoarding in an Afrikaner subpopulation (Lochner et al., 2005). Furthermore, in a recent cluster analytic study, the *COMT Met158Met* genotype was associated with higher scores on an obsessional/checking cluster, characterized by aggressive, sexual, religious and somatic obsessions and related checking compulsions (Lochner et al., 2008). Our results are in line with this study, suggesting that the *COMT Val158Met* genotype might be associated with lower somatic and sensory phenomena symptoms in females.

We also performed a category-based analysis, using predefined symptom categories as performed in previous factor analytic studies (Cavallini et al., 2002; Hasler et al., 2006; Kim et al., 2005), using principal components analysis with promax rotation. This factor analysis yielded a 4-factor structure: (1) Aggressive, sexual, religious, and somatic obsessions with checking compulsions, (2) contamination obsessions and cleaning compulsions, (3) Repeating, ordering and arranging compulsions, and (4) Hoarding obsessions and compulsions. These factors showed considerable overlap with four of the factors obtained by item-level factor analysis. No association was found between the

factors of the category-based factor analysis and the *COMT Val158Met* genotype or allele frequencies (data not shown). This might be the result of phenotypic heterogeneity of some of the category-based symptom dimensions. A recent study by Pinto et al. suggests that aggressive obsessions may be heterogeneous (Pinto et al., 2007).

Due to the heterogeneity of some of the YBOCS-CL categories, item-level factor analysis seems to be superior to category-based factor analysis with respect to homogeneity of symptom dimensions, and may increase the chances to find meaningful associations with genotypes. Our data seem to support this.

This study has several limitations. First, there were differences in YBOCS-severity scores, age of onset and prevalence of positive family history between the different collection sites. These differences may reflect differences in study methods used. Patients from the Utrecht site were diagnosed with current OCD whereas patients recruited in Amsterdam and Groningen had a lifetime diagnosis of OCD. Current rather than lifetime YBOCS-severity scores were reported. Further, a number of patients from the Amsterdam site were recruited within the scope of a project concerning Tourette's disorder, which explains the earlier age of onset at the Amsterdam site. This difference in recruitment strategies between sites causes heterogeneity in our study population. Although this may reduce the power to find correlations between genes and phenotype, this heterogeneity increases the generalizability of our findings.

A limitation of case-control studies in general is that they can be biased by population stratification (Thomas and Witte, 2002). Family-based approaches have been developed to circumvent this problem (Schulze and McMahon, 2002). Alternatively, multiple markers can be typed to test and correct for possible population stratification by genomic control or structured association methods (Pritchard and Donnelly, 2001). The use of these methods in future studies would be desirable. A further limitation of our case-control study is that the power to detect a sex-specific effect was limited.

A limitation of the use of symptom dimensions in genetic analysis could be that symptoms may change over time. However, symptom patterns in adults may be more stable than previously thought and changes in symptoms occur usually within rather than between symptom dimensions (Mataix-Cols et al., 2002b). Moreover, the YBOCS-CL is in itself not quantitative in nature. The dimensional YBOCS (Rosario-Campos et al., 2006), which has been designed to investigate the severity of symptoms in six symptom dimensions, could be used as a next step to refine the phenotype in genetic studies (Rosario-Campos et al., 2006).

Although the *COMT Val158Met* variant is an important functional polymorphism, this variant might not explain the whole variation in COMT activity (Chen et al., 2004). The *Val158Met* variant is not in complete linkage disequilibrium with other variants in the *COMT* gene and therefore does not capture the complete genetic variance in the *COMT*

gene (DeMille et al., 2002). DeMille et al. concluded in their study that haplotypes should be used in association studies for the *COMT* gene to have the greatest power to detect the effects of functional variation (DeMille et al., 2002). Therefore, future studies of the *COMT* gene in OCD using haplotypes including the *COMT Val158Met* polymorphism and other variants in the *COMT* gene such as variants in the estrogen responsive element located in the P2 promoter are indicated.

In conclusion, we investigated the role of the *COMT Val158Met* polymorphism on the phenotypic expression of OCD in a large cohort of patients. Our results suggest a sex dependent association between the *COMT Val158Met* polymorphism and OCD in males, which is in line with previous research. In addition, they suggest a possible role for the *COMT Val158Met* polymorphism in somatic obsessions and sensory phenomena in women.

Chapter 6

# The role of the BDNF Val66Met and COMT Val158Met polymorphism in symptom dimensions of Tic Disorders: Preliminary results

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**Abstract**

*Tourette's disorder (GTS) is a complex disorder with a multigenetic background. Candidate gene studies to date have yielded inconsistent results, possibly as a consequence of genetic and phenotypic heterogeneity of the disorder. To improve phenotypic homogeneity, we used tic symptom dimensions as phenotypes in 290 tic/GTS patients 535 controls, to study the role of the Brain Derived Neurotrophic Factor (BDNF) Val66Met and the Cathechol-O-methyl transferase (COMT) Val158Met polymorphisms.*

*Results: Exploratory and confirmatory item-based factor analysis on Yale Global Tic symptom checklist of GTS/tic disorder patients yielded both one factor and six factors to best fit the data. Internal consistency of these factors was reasonable. The BDNF 66Met allele and BDNF Met66Met genotype were associated with GTS/CMT in females. The COMT Met158Met genotype was associated with lower Yale Global Tic Severity Scale (YGTTSS) vocal tic severity, and in men, the COMT Val158Met genotype with lower Yale Brown Obsessive Compulsive Scale (YBOCS) obsession and total YBOCS severity. No significant associations were found between tic factor scale scores and genotypes or alleles of the BDNF Val66Met or the COMT Val158Met polymorphism. However, there was a trend for a higher score on a miscellaneous tic factor for female patients with the BDNF Val66Met genotype and a lower score for female patients with the BDNF Val66Val genotype compared to female patients with other BDNF genotypes.*

*Conclusions: These findings suggest 1) an association of the low functioning BDNF 66Met allele in women with tics, 2) a protective role for the_COMT Met158Met genotype on tics in all patients, and of the COMT Val158Met genotype on obsessive compulsive symptom severity in men with tic disorders.*

## Introduction

Tourette's Disorder (GTS) is characterized by multiple motor and one or more vocal tics (American Psychiatric Association, 1994). Family and twin studies strongly indicate a genetic background in the occurrence of GTS, with increased morbidity associated with bilineal transmission in up to 25% of cases (McMahon et al., 2003; Scahill et al., 2001).

The mode of transmission of GTS is likely to be complex and to involve multiple genes (Keen-Kim and Freimer, 2006; Pauls, 1992). Between 40-60% of GTS patients have co-morbid obsessive-compulsive symptoms (OCS), and family studies show that at least some forms of obsessive compulsive disorder (OCD) and GTS are related (Pauls, 1992; Robertson et al., 2002). Sex differences have been described in the transmission of GTS and co-morbid OCD within families, independent of sex of the proband, with OCD occurring in increased rates in female relatives, and tics in male relatives (Pauls, 1992). Furthermore, in a mouse model of GTS+OCD transgenic male mouse had more a severe tic phenotype (more tic flurries) compared to transgenic female mice (Nordstrom and Burton, 2002).

To date, candidate gene studies have generally yielded inconsistent results (Keen-Kim and Freimer, 2006; Pauls, 1992).

Recent neuroimaging studies show evidence for abnormalities of dopaminergic neurotransmission in the basal ganglia (Albin et al., 2003; Cheon et al., 2004; Serra-Mestres et al., 2004; Singer et al., 2002; Wong et al., 2008). A neuroimaging study by Albin et al (2003) suggested increased dopaminergic innervations in the right ventral striatum of GTS patients. Moreover, Singer et al. (2002) and Wong et al. (2008) pointed out that a hypersensitive phasic dopamine transmission probably exists in GTS. Therefore, genes in the dopaminergic pathway are suitable targets for candidate gene studies.

One such candidate is Catecholamine-*O*-methyl transferase (COMT), involved in the degradation of dopamine and norepinephrin in brain and body tissue. The *COMT* gene is located at chromosome 22q11 and contains a functional polymorphism causing an amino acid substitution at position 158 (*Val158Met*). The *Met158Met* genotype is associated with a 3-4 times reduced enzyme activity compared to the *Val158Val* genotype and the *Val158Met* genotype has intermediate activity (Lachman et al., 1996b). Three association studies investigated the *COMT Val158Met* polymorphism in GTS, with negative results (Cavallini et al., 2000; Lim et al., 2009; Tarnok et al., 2007). Moreover, one linkage study did not show linkage between the *COMT* gene and GTS (Barr et al., 1999b). However, associations have been described between the *COMT Val158Met* polymorphism and OCD (Alsobrook et al., 2002b; Denys et al., 2006a; Karayiorgou et al., 1997, 1999; Katerberg et al., 2009a; Niehaus et al., 2001; Pooley et al., 2007; Poyurovsky et al., 2005). Further, a

recent meta-analysis of case-control association studies in OCD showed evidence for an association between the *COMT 158Met* allele and OCD in males (Pooley et al., 2007).

Genes involved in neuronal growth may also warrant investigation in relation to GTS. One of these genes is the Brain Derived Neurortrophic Factor (*BDNF*) gene. BDNF has been implicated in neuronal survival and in activity–dependent neuroplasticity (Hennigan et al., 2007). The *BDNF* gene contains a polymorphism (G196A) that causes a valine to methionine substitution in the prodomain of the BDNF protein (*Val66Met*). This polymorphism has been shown to reduce activity-dependent BDNF secretion in transfected neurons (Egan et al., 2003). Studies in knockout mice as well as studies investigating B cell lines suggest that BDNF modulates serotonin transporter function (Daws et al., 2007; Mössner et al., 2000). Pharmacological and neurobiological studies indicate that the serotonergic system is involved in the pathogenesis of GTS, by a decreased serotonergic activity in the striatum which may facilitate phasic dopamine response (Heinz et al., 1998; Wong et al., 2008).

The *BDNF Val66Met* polymorphism has been investigated in only one family-based association study for GTS (Klaffke et al., 2006). There was no preferential transmission of any of the alleles. For OCD, eight association studies investigating the *BDNF Val66Met* have been published, using either a family-based approach (Dickel et al., 2007; Hall et al., 2003; Mössner et al., 2005; Zai et al., 2005b) or a case-control design (Alonso et al., 2008a; Hemmings et al., 2008; Katerberg et al., 2009c; Wendland et al., 2007). In the family-based studies, both an *under*transmission of the *BDNF Met66* allele in OCD (Hall et al., 2003), and the association of a haplotype including the *BDNF Val66* allele with reduced risk for OCD has been reported (Alonso et al., 2008a). In the case-control studies an association was found of the *BDNF Met66* allele with OCD in males (Hemmings et al., 2008), and of later age of onset and a less frequent positive family history for OCS in OCD females with the *BDNF Met66Met* genotype (Katerberg et al., 2009c). Moreover, in that study a trend was found for a sexual and religious obsessions symptom dimension in patients with the *BDNF Val66Val* genotype.

The inconsistent results described above may be due to genetic and/or symptomatic heterogeneity. Identification of homogeneous symptom dimensions by factor analytic or cluster analytic approaches seems promising, especially when item-level based analyses are applied (Alonso et al., 2008a; Cavallini et al., 2000; Katerberg et al., 2009a, 2009b; Kim et al., 2005, 2009; Wendland et al., 2007). These approaches have been extensively used for OCD. For GTS such strategies are still scarce, and to our knowledge, studies combining symptom dimensions with candidate gene studies are lacking.

A recent cluster analysis of tic symptoms in two genetically isolated populations has found that GTS patients could be grouped into two clusters according to their tics (Mathews et al.,

2007a). The first cluster consisted of patients with predominantly simple tics; the second cluster was characterized by persons with multiple complex tics and OCS. Patients in the second cluster had increased motor and phonic tic severity and global impairment and were more often treated with antipsychotic medication or multiple treatments.

One previous factor analytic study in GTS, using predefined symptom categories, yielded four factors: 1) aggressive phenomena, 2) purely motor and phonic tic symptoms, 3) compulsive phenomena, and 4) tapping and the absence of grunting (Alsobrook and Pauls, 2002). High intraclass correlations were found within families for factor 1, 2, and 4, suggesting these factors may comprise heritable components of GTS. High scores on factor 1 and 3 were associated with increased rates of comorbid attention deficit hyperactivity disorder (ADHD). Male patients had higher scores on factor 2. Factor 3 scores were associated with OCD in relatives and with age of GTS onset. Interestingly, factor 3 was not associated with a diagnosis of co-morbid OCD. None of the factors were associated with presence of GTS in relatives. A similar approach in a large multigenerational family yielded 3 factors: 1) predominantly 'pure' tics 2) predominantly ADHD and aggressive behaviors, and 3) predominantly 'depression-anxiety-obsessional symptoms and self-injurious behaviors' (Robertson and Cavanna, 2007).

Robertson et al. (2008) performed hierarchical agglomerative cluster analysis on data of 32 tic symptoms of 410 unrelated patients and subsequently performed principal component analysis on the resulting cluster scores. Five factors were found: 1) complex and self injurious behaviors, 2) complex motor tics; 3) simple motor and vocal tics; 4) compulsive-like behaviors, and 5) simple tics and touching. Factor 1 scores were associated with a lower age of tic onset. All factors except factor 5 were associated with co-morbid OCD. None of the factors were associated with family history of OCD. Females tended to have lower factor scores for all factors.

The current study has aimed at extending the findings described here-above investigating symptom dimensions in GTS, by identifying probable symptom dimensions through item-level factor analysis on the Yale Global Tic Severity Scale (YGTSS) symptom checklist items (Leckman et al., 1989). The resulting symptom dimensions were investigated 1) on their relation to clinical characteristics (family history, sex, tic severity, and comorbid OCD) 2) their association with the *BDNF Val66Met* variant and the *COMT Val158Met* polymorphism. In addition, correlations between genotypes and phenotypic characteristics such as tic and OC symptom severity, and family history of tics were investigated.

## Patients and methods

*Participants*

This project encompasses a joint venture between the Department of Psychiatry of VU University Medical Center (VUMC) and the Department of child and adolescent Psychiatry of the University Medical Center Groningen (UMCG) in the Netherlands. Patients were recruited from the outpatient Clinic for Anxiety Disorders, GGZ Buitenamstel in Amsterdam, and the department of child and adolescent psychiatry of the University Medical Center Groningen (The Netherlands). Patients with a definite or probable diagnosis for tic disorders according to DSM-IV criteria (American Psychiatric Association, 1994) were included in the study. The study was approved by the Medical Ethical Review Boards of the participating centers. All patients or (in case of minors) their parents as well as participating family members gave written informed consent for participation in the study.
Control subjects encompassed an unscreened convenience sample recruited from the general population.

*Clinical assessment*

Tic diagnosis was established with the aid of the Diagnostic Confidence index (DIC;Robertson et al., 1999). Current tic symptoms and severity were assessed using the YGTSS (Leckman et al., 1989). The YGTSS encompasses a symptom checklist and a 10 item tic severity interview, each item being scored between 0 and 5. Vocal and motor tics are scored separately on frequency, intensity, and interference (Leckman et al., 1989). Family history of tics was determined, and considered to be positive if the presence of tics was reported in at least one first degree family member. The presence of OCD was established according to DSM-IV criteria using the Structured Clinical Interview for Axis I disorders (SCID-I/P) (First et al., 1998). Current severity of the OCS was assessed using the Yale-Brown Obsessive Compulsive Severity Scale (YBOCS) or Children's YBOCS (CYBOCS) (Goodman, 1989a, 1989b). The YBOCS is a 10 item interview with separate subscales for time spent, interference, resistance and control of obsessions (items1-5) and compulsions (items 6-10).

*Genotyping*

DNA was isolated from blood using a chloroform/isopropanol extraction method (Meulenbelt et al., 1995) from buccal swabs using a salting out procedure (Miller et al., 1988), or from sputum using an Oragene DNA self collection kit (DNA Genotek Inc., Ottawa, Canada) according to manufacturers instructions. Samples were genotyped for the *BDNF Val66Met* genotype in a SNPlex™ genotyping assay (Applied Biosystems, Foster

City, CA, USA) or by Taqman© genotyping assay (Applied Biosystems, Foster City CA, USA: Assay on demand, ID CD_11592758_10) according to manufacturer's instructions and for the *COMT Val158Met* polymorphism using a Taqman® Drug metabolism genotyping assay (number C__25746809_50) according to manufacturer's instructions using a 5 or 2 µl final reaction volume.

*Statistical analysis*

Power calculations were performed with the genetic power calculator (Purcell et al., 2003) assuming a disease prevalence of 0.5% (Keen-Kim and Freimer, 2006) using the allele frequencies observed in our control population. Hardy-Weinberg equilibrium was tested using chi square tests in Microsoft Office Excel. Genotype and allele frequencies were compared between patients and controls and between patients with different phenotypes using Fisher's exact tests. Missing values in the YBOCS and YGTSS were imputed with Solas™ 3.2 (statistical solutions, ltd; Cork, Ireland), using predictive model-based imputation. Five different datasets were imputed and mean results of these datasets were used in the final analysis. Since data on most continuous variables such as YBOCS and mean score per item were not normally distributed, differences in these variables between patients with different genotypes and between the different alleles of the *BDNF Val66Met* and the *COMT Val158Met* polymorphism were examined using non-parametric tests (Kruskal-Wallis and Mann-Whitney *U* tests).

*Factor analysis*

Explorative item-by-item level factor analysis with promax rotation was performed using MPlus (Muthén and Muthén, 1998-2006). Subsequently, confirmatory factor analysis for categorical variables was performed to establish the number of factors and factor constitution using the following fit indices: the $\chi 2$ statistic, the comparison of fit index (CFI), the Tucker-Lewis Index (TLI), and the Root Mean Square Error of Approximation (RMSEA). Values of the CFI and of TLI approaching 0.95, and values of the RMSEA $<$ 0.05 are generally indicative of a good fit (Browne and Cudek, 1993; Hu and Bentler, 1999). Internal consistency of the factors was examined using Cronbach's alpha. Items were assigned to the factor(s) on which they had loadings $\geq 0.4$ or on the factor on which they had the highest loading. Both the explorative and the confirmatory factor analysis were based on dichotomous variables.

Mean item scores for each of the obtained factors (factor scale scores) were calculated for each patient, representing the prominence of this factor in the patient. Kruskal-Wallis tests and Mann-Whitney *U* tests were performed to investigate whether mean item scores per factor were associated with the *BDNF Val66Met* or *COMT Val158Met* genotype or alleles. All statistical tests were performed using the Software Package for Social Sciences (SPSS)

version 14.1 or 16.0 (SPSS Inc, Chicago, IL). Eleven phenotypes were tested (i.e. absence/presence of OCD, YGTSS score, YBOCS severity, tic family history, OCD, and tic symptom dimensions). Associations between mean factor scores and other clinical variables, (gender, YGTSS scale scores, family history, OCD and YBOCS severity) were investigated. To control for multiple testing, Bonferroni correction was applied, with a p-value of 0.05/11 =0.0045 considered significant.

*Sample size and power considerations*

Based on the allele frequencies in our combined control population (see table 5), the power to detect a dominant effect of the *BDNF Met66* allele with a relative risk of 2 for the case-control study was 97.2% for the total sample, 84.5% for males, and 65.4% for females, respectively. The power to detect a recessive effect of the *BNDF Met66* allele with a relative risk of 2 for GTS was 22.2% in the total sample, 15.2% in males, and 11.1% in females. The power to detect a 2 fold increased risk of the *COMT 158Met* allele in a dominant model was 58.7%, 45.6%, and 22.2% for all patients, males, and females respectively. The power to detect a relative risk of 2 for the *COMT 158Met* allele in a recessive model was 88.8% for all patients, 64.0% for males, and 52.5% for females.

**Results**

Two hundred and seven probands were included in the genetic association study (140 males, 67 females: mean age at assessment was 28.2±15.3 years). Demographic characteristics of this group are summarized in table 1. Mean total score on the YGTSS was 18.2±10.6 and mean scores on the motor and vocal subscales were 11.3±5.8 and 6.9±5.9 respectively, representing mild-to-moderate tic severity. Mean total YBOCS/CYBOCS score was 7.9±9.1. Patients from Groningen were younger, and had lower motor YGTSS tic scores as well as lower YBOCS/CYBOCS severity scores than patients from Amsterdam. Men had higher scores on the vocal subscale of the YGTSS and the compulsion subscale of the YBOCS compared to women. Thirty three percent had a diagnosis of comorbid OCD; sixty eight percent had a positive family history of tics. Yale tic symptom checklist data were available of 125 of these patients, 61 affected family members, and an additional 104 affected subjects from 100 families, yielding 290 patients with tic disorders from 226 families (252 patients with definite or probable GTS, 27 patients with definite or probable Chronic Motor Tic disorder (CMT), 3 patients with definite or probable Chronic Vocal Tic disorder (CVT), and 8 patients with definite or probable CMT and CVT). Demographic characteristics of these probands included in the factor analysis are summarized in table 1. Mean total score on the YGTSS was 16.4±10.2 and mean scores on the motor and vocal subscales were 10.4±6.0 and 6.0±5.6 respectively, representing mild-to-moderate tic severity. Mean total YBOCS/CYBOCS score was 6.4±8.3. Patients from Groningen were younger, and had lower motor and total YGTSS tic scores as well as lower YBOCS/CYBOCS obsessions severity scores than patients from Amsterdam. Men had higher scores on the total YGTSS as well as the motor and vocal subscores of the YGTSS compared to women (data not shown). Eighteen percent of the patients had a diagnosis of comorbid OCD. Sixty-four percent of the patients had a positive family history of tics.

Table 1. Demographic and clinical characteristics of the study groups.

| | Amsterdam/ Rotterdam | Groningen | Total | χ2/Z* | p | χ2/Z** | p |
|---|---|---|---|---|---|---|---|
| Cohort included in genetic studies | | | | | | | |
| N | 152 | 55 | 207 | | | | |
| Female (%) | 54 (35.5%) | 13 (23.6%) | 67 (32.4%) | 2.608 | 0.131 | | |
| Age | 31.1±15.4 (n=145) | 20.5±12.4 (n=55) | 28.2±15.3 | -4.478 | **<0.001** | -1.845 | 0.065 |
| YGTSS motor score | 11.9±5.8 (n=123) | 9.9±5.7 (n=52) | 11.3±5.8 | -2.265 | **0.024** | -0.106 | 0.915 |
| YGTSS vocal score | 7.1±5.7 (n=123) | 6.4±6.5 (n=52) | 6.9±5.9 | -1.041 | 0.298 | -2.164 | **0.030** |
| YGTSS total score | 19.0±10.2 (n=123) | 16.3±11.4 (n=52) | 18.2±10.6 | -1.934 | 0.053 | -1.224 | 0.221 |
| YBOCS obsessions score | 4.4±5.0 (n=106) | 1.5±3.1 (n=47) | 3.5±4.7 | -3.651 | **<0.001** | -0.465 | 0.642 |
| YBOCS compulsions score | 5.5±5.2 (n=106) | 2.0±3.9 (n=47) | 4.4±5.1 | -3.979 | **<0.001** | -2.856 | **0.004** |
| YBOCS total score | 9.9±9.4 (n=106) | 3.4±6.4 (n=47) | 7.9±9.1 | -4.226 | **<0.001** | -1.945 | 0.052 |
| Positive family history | 93 (67.9%; n=137) | 8 (57.1%; n=14) | 101(66.9%) | 0.662 | 0.552 | 0.002 | 1.000 |
| Comorbid OCD. | 43 (33.3%; n=129) | NA | 43 (33%) | | | 0.154 | 0.700 |
| Cohort included in factor analyses | | | | | | | |
| N | 200 | 90 | 290 | | | | |
| Female (%) | 73 (36.5%) | 11 (13.1%) (n=84) | 84 (29.6%) | 15.557 | **<0.001** | | |
| Age | 30.8±17.4 (n=200) | 12.3±3.3 (n=84) | 25.3±17.0 | -8.392 | **<0.001** | | |
| YGTSS motor score | 9.7±6.3 (n=192) | 12.0±4.9 (n=84) | 10.4±6.0 | -2.703 | **0.002** | | |
| YGTSS vocal score | 5.7±5.5 (n=192) | 6.5±5.6 (n=84) | 6.0±5.6 | -1.271 | 0.347 | | |
| YGTSS total score | 15.5±10.6 (n=192) | 18.5±8.9 (n=84) | 16.4±10.2 | -1.780 | **0.011** | | |
| YBOCS obsessions score | 3.2±4.5 (n=168) | 1.7±3.7 (n=84) | 2.7±4.3 | -3.157 | **0.007** | | |
| YBOCS compulsions score | 3.9±4.9 (n=168) | 3.1±4.3 (n=83) | 3.7±4.7 | -0.941 | 0.204 | | |
| YBOCS total score | 7.2±8.8 (n=168) | 4.8±6.8 (n=83) | 6.4±8.3 | -2.559 | 0.075 | | |
| Positive family history | 142 (75.9%; n=184) | 27 (35.1%; n=77) | 169 (64.0%) | 38.421 | **<0.001** | | |
| OCD as a codiagnosis | 43 (23.1%; n=186) | 2 (3.4%; n=58) | 45 (18.4) | 11.373 | **<0.001** | | |

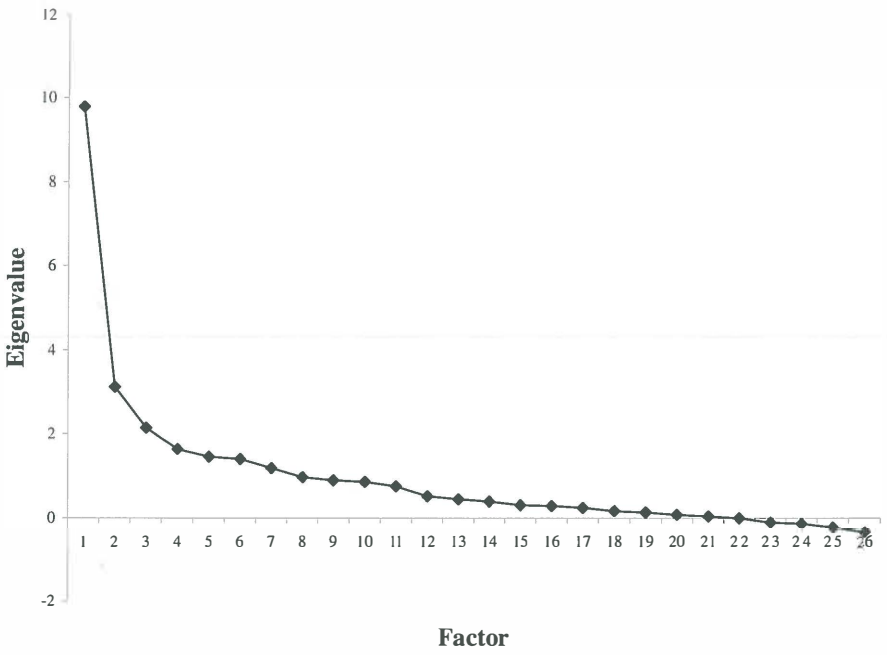
YGTSS: Yale Global Tic Severity Scale; YBOCS = Yale-Brown Obsessive Compulsive Scale; OCD= Obsessive compulsive disorder; NA= Not available.

*Patients from Amsterdam/Rotterdam versus patients from Groningen

**Male patients versus female patients

Factor analysis

Of the Yale tic symptom checklist used in the analyses, 3 items ("other single vocal tics", "other simple motor tics", and "other pattern of complex motor tics") were excluded since these items were considered to be too heterogeneous. Explorative item-level factor analysis yielded 7 factors with an eigenvalue >1. The scree plot of the item-level factor analysis (figure I) supported a one factor model.




Subsequently, confirmatory factor analysis was performed to compare models with 1 to 7 factors. A six factor model showed the best fit (see table 2): Factor 1: Complex and self-injurious tics (shoulder, arm and stomach movements, complex vocal tics and self injurious behavior); Factor 2: Compulsion factor (Complex motor tics and tic-related compulsions); Factor 3: Common simple tics factor (Throat clearing and eye tics); Factor 4: Complex vocal tic factor (Whistling, animals and bird noises, syllables, mouth and nose tics); Factor 5: Common complex tics factor (Facial, head and neck tics with coughing, sniffing and syllables); and Factor 6: Miscellaneous factor (Complex shoulder movements and echolalia).

Table 2. Fit indices of the 1 to 6 factor models obtained by item-level factor analysis of the Yale Tic Severity Scale symptom checklist*.

| | 1-factor model | 2-factor model | 3-factor model | 4-factor model | 5-factor model | 6-factor model | 7-factor model |
|---|---|---|---|---|---|---|---|
| $\chi 2$ | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| CFI | 0.721 | 0.758 | 0.768 | 0.820 | 0.837 | 0.868 | 0.870 |
| TLI | 0.793 | 0.822 | 0.830 | 0.870 | 0.881 | 0.877 | 0.902 |
| RMSEA | 0.130 | 0.120 | 0.118 | 0.102 | 0.098 | 0.088 | 0.088 |

CFI= Comparison of fit index, TLI= Tucker-Lewis Index (TLI), RMSEA = Root Mean Square Error of Approximation. *Values of the CFI and of TLI approaching 0.95, values approaching 0.08 of the SRMR and values of the RMSEA < 0.05 are generally indicative of a good fit.

Factor structure and factor loadings are shown in table 3.

Cronbach's alpha for the different factors and Pearson's correlation coefficients between the different factors are summarized in table 4. Cronbach's alpha coefficients were all above 0.640 (range 0.643-0.794), indicating a reasonable internal consistency of the factors. Correlations between sex, family history of tics, comorbid OCD, YGTSS scores and YBOCS scores are summarized in table 5. There were no sex differences in factor scale scores. A positive family history for tics was correlated with a lower factor scale score for factor 2 (the compulsions factor; Z= -2.857 p = 0.004), and factor 5 (common complex tic factor; Z=-3.575, p = <0.001). Patients with comorbid OCD had higher mean factor scale scores on factor 1, 2, and 4 (p all≤ 0.003), a lower score on factor 6 (miscellaneous factor; Z=-2.920, p=0.003), and a trend for a higher score for factor 5 (Z=-1.991; p=0.046). Factor scale scores of all factors were significantly correlated with scores on the YGTSS total and motor and vocal subscales scores (all p≤ 0.004) except for the correlation between factor 4 (complex vocal tic factor) with the vocal subscale. There was a positive correlation between mean score per items and the YBOCS severity score except for the common single tic factor (factor 3).

| | Factor 1 Complex and self-injurious tics | Factor 2 Compulsion factor | Factor 3 Common simple tics | Factor 4 Complex vocal tics | Factor 5 Common complex tics | Factor 6 Miscellaneous Factor |
|---|---|---|---|---|---|---|
| Single movements with arm or hand | **0.836** | 0.047 | -0.027 | -0.019 | 0.087 | -0.023 |
| Singe movement with leg, foot or toe | **0.720** | 0.258 | 0.163 | -0.274 | -0.021 | 0.058 |
| Single shoulder movements | **0.632** | -0.218 | 0.365 | -0.171 | 0.232 | 0.106 |
| Palilalia | **0.566** | -0.034 | -0.050 | 0.327 | -0.114 | 0.262 |
| Self injurious behavior | **0.544** | 0.206 | -0.059 | 0.206 | 0.070 | -0.220 |
| Words | **0.515** | -0.125 | 0.226 | 0.260 | -0.032 | 0.240 |
| Complex vocal tics, other speech problems | **0.396** | 0.316 | 0.097 | 0.276 | -0.349 | 0.202 |
| Single movements with the stomach | **0.340** | 0.204 | 0.219 | 0.102 | 0.106 | -0.021 |
| Unusual positions | -0.054 | **0.898** | -0.149 | 0.209 | -0.034 | 0.000 |
| Bending or rotating | 0.099 | **0.750** | -0.225 | 0.020 | 0.026 | 0.001 |
| Complex movements with leg, foot or toe | -0.038 | **0.619** | -0.192 | -0.373 | 0.318 | 0.204 |
| Turn or stretch | -0.189 | **0.600** | 0.095 | 0.187 | 0.014 | 0.283 |
| Complex movements with arm/hand | 0.190 | **0.556** | -0.049 | -0.156 | 0.036 | 0.230 |
| Tic-related compulsive acts | 0.300 | **0.511** | 0.032 | -0.011 | -0.037 | 0.031 |
| copropraxia | 0.262 | **0.421** | 0.030 | 0.069 | 0.013 | -0.335 |
| Eye tics | 0.066 | -0.157 | **0.758** | 0.018 | 0.072 | -0.067 |
| Throat clearing | 0.126 | -0.228 | **0.692** | 0.247 | 0.048 | 0.102 |
| Whistling | 0.036 | 0.117 | 0.286 | **0.816** | -0.163 | -0.007 |
| Syllables | 0.237 | -0.076 | -0.347 | **0.776** | **0.483** | 0.137 |
| Animal or bird noises | -0.172 | 0.075 | 0.210 | **0.775** | 0.027 | 0.143 |
| Mouth and nose tics | -0.015 | 0.362 | 0.390 | **0.442** | 0.265 | -0.231 |
| Head/neck tics | 0.297 | -0.075 | -0.055 | 0.221 | **0.887** | -0.027 |
| Coughing, sniffing | -0.155 | 0.203 | 0.353 | -0.239 | **0.663** | 0.071 |
| Facial tics | -0.085 | 0.158 | 0.315 | 0.104 | **0.551** | 0.046 |
| Complex shoulder movements | 0.047 | 0.167 | -0.008 | 0.100 | 0.059 | **0.839** |
| Echolalia | 0.269 | 0.074 | 0.005 | 0.131 | 0.032 | **0.649** |

Table 4 Standardized Cronbach's alpha and correlations between the different factors of the six factor model obtained by item-level factor analysis of the Yale Tics Severity Checklist.

| | **Factor 1** | **Factor 2** | **Factor 3** | **Factor 4** | **Factor 5** | **Factor 6** |
|---|---|---|---|---|---|---|
| Cronbach's alpha | 0.794 | 0.674 | 0.643 | 0.679 | 0.648 | 0.761 |
| Factor 2 | 0.423 | | | | | |
| Factor 3 | 0.407 | 0.153 | | | | |
| Factor 4 | 0.547 | 0.291 | 0.499 | | | |
| Factor 5 | 0.319 | 0.368 | 0.272 | 0.284 | | |
| Factor 6 | 0.556 | 0.453 | 0.311 | 0.409 | 0.357 | |

Genetic studies

Genotypes and allele frequencies for the cases and controls are summarized in table 6. Genotypes of the *BDNF Val66Met* polymorphism were available for 203 unrelated patients and 535 controls; genotypes of the *COMT Val158Met* polymorphism were available for 201 unrelated patients and 310 controls. Genotypes for both polymorphisms were in Hardy Weinberg equilibrium for all groups. Since there was no difference in *COMT Val158Met* genotype ($\chi2=0.881$; $p=0.675$) or allele frequency ($\chi2=0.877$; $p=0.371$) nor in *BDNF Val66Met* genotype ($\chi2=0.522$; $p=0.760$) or allele frequency ($\chi2=0.193$; $p=0.704$) between patients from Groningen and Amsterdam, patients from both sites were pooled for analysis.

Genotype-phenotype associations for the *BDNF Val66Met* polymorphism

There was an increased frequency of the *BDNF 66Met* allele ($\chi2$=9.552; p=0.002) and the *BDNF Met66Met* ($\chi2$=10.513; p=0.005) genotype in patients compared to controls. Stratification by gender showed that this difference was confined to the females.

There was no difference in the rate of positive family history or comorbid OCD between different genotypes or alleles of the *BDNF Val66Met* polymorphism. There was no association between YGTSS severity scores and genotypes or alleles of the *BDNF Val66Met* polymorphism.

There were no differences in mean factor scale scores between patients with different *BDNF Val66Met* genotypes. However, there was a trend for a higher factor 6 score in female patients with the *BDNF Val66Met* compared to female patients with other *BDNF* genotypes (Z=-2.302; p = 0.021) and a lower factor 6 score in patients with the *BDNF Val66Val* genotype compared to patients with other *BDNF* genotypes (Z=-2.302; p = 0.021).

Genotype-phenotype correlations for the *COMT Val158Met* polymorphism.

There was no difference in genotype ($\chi2$=0.641; p=0.728) or allele frequency ($\chi2$=0.618; p=0.442) for the *COMT Val158Met* polymorphism between cases and controls nor were there differences in genotype or allele frequencies between cases and controls after stratification by gender. There was no difference in the rate of positive family history or comorbid OCD between different genotypes or alleles of the *COMT Val158Met* polymorphism, nor were there any differences in mean factor scale scores between patients with different *COMT Val158Met* genotypes.

Patients with the *COMT Met158Met* genotype had a lower YGTSS vocal score (Z=-2.894; p=0.004) and a trend for a lower total YGTSS score (Z=-2.209; p=0.027) compared to patients with other genotypes. Patients with the *COMT Val158Met* genotype showed a trend for a higher YBOCS compulsion score compared to patients with other *COMT Val158Met* genotype. Male patients with the *COMT Val158Met* genotype had a lower YBOCS total severity score (Z=-2.471; p=0.013) with lower YBOCS obsessions score (Z=-2.379; p=0.017) compared to male patients with other *COMT Val158Met* genotypes at a trend level.

Table 5. Correlation of factor scale scores with other clinical characteristics.

| | | Factor1 | Factor2 | Factor3 | Factor4 | Factor 5 | Factor6 |
|---|---|---|---|---|---|---|---|
| Male (n=200) | | 0.384±0.293 | 0.209±0.218 | 0.773±0.343 | 0.454±0.318 | 0.474±0.332 | 0.265±0.394 |
| Female (n= 84) | | 0.451±0.330 | 0.209±0.205 | 0.756±0.384 | 0.461±0.342 | 0.441±0.351 | 0.298±0.419 |
| | Z | -1.565 | -0.212 | -0.023 | -0.099 | -0.743 | -0.531 |
| | p | 0.118 | 0.832 | 0.982 | 0.922 | 0.457 | 0.595 |
| OCD (n=45) | | 0.625±0.286 | 0.359±0.206 | 0.789±0.377 | 0.661±0.267 | 0.550±0.309 | 0.467±0.470 |
| No OCD (n=199) | | 0.386±0.299 | 0.180±0.233 | 0.769±0.358 | 0.449±0.334 | 0.440±0.349 | 0.256±0.395 |
| Z | Z | -4.639 | -5.021 | -0.614 | -3.950 | -1.991 | -2.920 |
| p | p | **<0.001** | **<0.001** | 0.539 | **<0.001** | **0.046** | **0.003** |
| Positive family history (n=95) | | 0.405±0.307 | 0.189±0.219 | 0.743±0.378 | 0.416±0.339 | 0.410±0.344 | 0.302±0.434 |
| Negative family history (n =169) | | 0.442±0.303 | 0.253±0.208 | 0.816±0.301 | 0.473±0.294 | 0.561±0.304 | 0.237±0.349 |
| Z | Z | -0.978 | -2.857 | -1.229 | -0.518 | -3.575 | -0.667 |
| p | p | 0.328 | **0.004** | 0.219 | 0.604 | **<0.001** | 0.505 |
| YBOCS obsessions score | r | 0.304 | 0.286 | 0.104 | 0.295 | 0.184 | 0.196 |
| | p | **<0.001** | **<0.001** | <0.099 | **<0.001** | **0.003** | **0.002** |
| YBOCS compulsions score | r | 0.352 | 0.357 | 0.068 | 0.265 | 0.167 | 0.153 |
| | p | **<0.001** | **<0.001** | 0.281 | **<0.001** | **0.008** | **0.015** |
| YBOCS total score | r | 0.360 | 0.355 | 0.095 | 0.306 | 0.193 | 0.192 |
| | | **<0.001** | **<0.001** | 0.133 | **<0.001** | **0.002** | **0.002** |
| YGTTS motor score | r | 0.376 | 0.380 | 0.286 | 0.214 | 0.458 | 0.257 |
| | p | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| YGTTS vocal score | r | 0.263 | 0.219 | 0.205 | 0.088 | 0.276 | 0.154 |
| | | **<0.001** | **<0.001** | **0.001** | 0.145 | **<0.001** | **0.011** |
| YGTTS total score | r | 0.362 | 0.341 | 0.278 | 0.172 | 0.417 | 0.233 |
| | p | **<0.001** | **<0.001** | **<0.001** | **0.004** | **<0.001** | **<0.001** |

Table 6. Genotype and allele frequency distribution.

| | n | Val/Val | Val/Met | Met/Met | p | Val | Met | p |
|---|---|---|---|---|---|---|---|---|
| BDNF genotype | | | | | | | | |
| TD patients | 203 | 113 (55.7%) | 74 (36.5%) | 16 (7.9%) | | 300 (73.9%) | 106 (26.1%) | |
| Controls | 535 | 352 (65.8%) | 166 (31.0%) | 17 (3.2%) | **0.005** | 870 (81.3%) | 200 (18.7%) | **0.002** |
| Male TD patients | 139 | 82 (59.4%) | 49 (35.5%) | 7 (5.1%) | | 213 (77.2%) | 63 (22.8%) | |
| Male controls | 249 | 162 (65.1%) | 81 (32.5%) | 6 (2.4%) | 0.262 | 405 (81.3%) | 93 (18.7%) | 0.104 |
| Female TD patients | 65 | 31 (47.7%) | 25 (38.5%) | 9 (13.8%) | | 87 (66.9%) | 43 (33.1%) | |
| Female controls | 286 | 190 (66.4%) | 85 (29.7%) | 11 (3.8%) | **0.002** | 465 (81.3%) | 107 (18.7%) | **<0.001** |
| COMT genotype | | | | | | | | |
| TD patients | 201 | 46 (22.6%) | 95 (47.3%) | 60 (29.9%) | | 187 (46.5%) | 215 (53.5%) | |
| Controls | 310 | 77 (24.8%) | 150 (48.4%) | 83 (26.8%) | 0.728 | 304 (49.0%) | 316 (51.0%) | 0.442 |
| Male TD patients | 136 | 29 (21.3%) | 70 (51.5%) | 37 (27.2%) | | 128 (47.1%) | 144 (52.9%) | |
| Male controls | 156 | 44 (28.2%) | 77 (49.4%) | 35 (22.4%) | 0.354 | 165 (52.9%) | 147 (47.1%) | 0.184 |
| Female GTS patients | 65 | 17 (26.2%) | 25 (38.5%) | 23 (35.4%) | | 59 (45.4%) | 71 (54.6%) | |
| Female controls | 154 | 33 (21.4%) | 73 (47.4%) | 48 (31.2%) | 0.460 | 139 (45.1%) | 169 (54.9%) | 1.000 |

**Discussion**

To our knowledge, this is the largest study in GTS investing the *COMT Val158Met* or the *BDNF Val66Met* polymorphism, using tic symptom dimensions as relevant phenotypes.

The scree plot of the explorative item-level factor analysis of the Yale Tic symptom checklist supported a one factor model. This suggests that there might be one underlying etiological factor operant across symptom dimensions. The additional six factor solution showing a reasonable fit suggests that specific etiological factors might be involved in specific tic symptom dimensions. The six factor solution comprised: Factor 1: Complex and self-injurious tics; Factor 2: Compulsion factor (Complex motor tics and tic-related compulsions); Factor 3: Common simple tics factor (Throat clearing and eye tics); Factor 4: Complex vocal tic factor; Factor 5: Common complex tics factor (Facial, head and neck tics with coughing, sniffing and syllables), and Factor 6: Miscellaneous factor (Complex shoulder movements and echolalia)

Our study differs with respect to previous studies. First, in instruments used to collect data concerning tic symptoms differ. Moreover, Robertson and Cavanna included symptoms of other psychopathology in addition to tic symptoms in their analyses. Therefore, the composition and number of the symptoms included in the different studies differ. Second, previous studies used hierarchical agglomerative cluster analysis and principal component analysis on the resulting clusters, whereas this study used item-level factor analysis.

However, the six factor model of this study is by and large in line with previous studies (Mathews et al., 2007a; Robertson and Cavanna, 2007). In line with Mathews et al., who found two clusters of tics, one cluster with simple tics and on cluster with complex tics, the factors of this study can be decomposed into factors with mainly complex tics: factor 1 (complex tics, aggressive behavior and self-injurious behavior), factor 2 (complex motor tics and tic-related compulsions), and factor 6 (complex shoulder movements and echolalia); and factors with mainly single tics: factor 3 (throat clearing and eye tics), and factor 4 (whistling, mouth and nose tics and animal and bird noises). Therefore, simple tics and complex tics seem to constitute different factors.

That none of the factors was associated with a positive family history of tics is in accordance with the studies by Alsobrook et al. (2002a) and Roberson et al (2008). Interestingly, in our study, higher factor scale scores for two of the factors (the compulsion factor and the complex tics factor) were associated with a negative family history for tics. Future studies investigating the heritability of the factors identified should be performed using a twin or family-based approach.

Mean item scores of four of the factors were significantly associated with comorbid OCD and a fifth factor was associated with OCD at a trend level. Mean scores per item on factor

3 (Throat clearing and eye tics) were not associated with OCD. This is in concordance with the results of the study of Roberson et al (2005) in which 4 of 5 factors were found to be associated with OCD. OCD was also associated with the YGTTS and its vocal and motor subscales. These finding suggest that OCD is associated with more diverse and/or severe tics.

It is difficult to understand how complex shoulder movements and echolalia should constitute a biologically and clinically meaningful subtype of GTS. This could be an artifact caused, although supported by the confirmatory factor analysis, by retaining too much factors.

A limitation of the study is that lifetime symptom scores were used in the factor analysis. Lifetime symptoms may be subject to recall-bias. However, since tic severity tends to decline with age, current tic symptoms would probably not reflect the diversity of the tics in a single patient, especially in older patients. As a further limitation it should be mentioned that the Yale Tic Severity Scale symptom checklist is not quantitative in nature. Alternatively, quantitative approaches to tic symptom dimensions could be developed, similar to the Dimensional YBOCS for OCD (Rosario-Campos et al., 2006).

In this case-control study, we found an association between the *BDNF Val66Met* allele and GTS in females. The fact that this association was not found in transmission disequilibrium tests (Klaffke et al., 2006; own data) may be due to the fact that these family-based approaches used a small sample, with the consequence of limited power to detect differences. Hemmings et al (2008) reported an association between OCD and the *BDNF Met* allele in males. Hall et al., (2003) found an undertransmission of the *BDNF 66Met* allele in OCD. Other studies found no evidence of association between the *BDNF Val66Met* polymorphism and OCD (Alonso et al., 2008a; Dickel et al., 2007; Katerberg et al., 2009c; Wendland et al., 2007; Zai et al., 2005b). We did not replicate the association found between the *BDNF 66Met* allele and OCD in our cohort of patients with tic disorders. Previous research by Nemoto et al. (2006) suggested that the *BDNF Val66Met* polymorphism affects age-related brain morphology and that female carriers showed more age-related volume reduction in the Dorsolateral Prefontal Cortex. The authors hypothesized that these differences in age-related changes may be caused by changes in neuroprotective and stress resistant effects of BDNF caused by the *BDNF Val66Met* variant (Nemoto et al., 2006). Thinning of sensorimotor cortices in older children and adolescents with GTS have been described, correlated with worst ever tic severity and simple facial tics (Sowell et al., 2008). Therefore, one might hypothesize that the sex-specific association for the *BDNF 66Met* allele in females might be mediated by age-related changes of the brain.

Future studies to elucidate the influence of the *BDNF Val66Met* genotype on changes in brain anatomy in Tourette patients should be performed.

A limitation of case-control association studies is that these can be biased by population stratification (Thomas and Witte, 2002). Therefore, we cannot rule out the possibility that the difference between the case-control and family-based association study of the *BDNF Val66Met* polymorphism has been caused by population stratification. Multiple markers can be typed to test and correct for possible population stratification by genomic control or structured association methods (Pritchard and Donnelly, 2001). The use of these methods in future control studies would be desirable.

The lack of an association in the present study between the *COMT Val158Met* polymorphism and GTS is in accordance with previous studies (Barr et al., 1999b; Cavallini et al., 2000; Lim et al., 2009; Tarnok et al., 2007). An association between the *COMT 158Met* alleles and OCD has been described in males (Denys et al., 2006a; Karayiorgou et al., 1997; Karayiorgou et al., 1999; Pooley et al., 2007). In accordance with previous studies in patients with GTS, we did not find an association between the *COMT Val158Met* polymorphism and OCD in this sample of patients with TD (Cavallini et al., 2000). Some OCD patients who do not respond to serotonin reuptake inhibitor (SRRi) monotherapy benefit from the addition of antipsychotics, especially patients with comorbid tics (Bloch et al., 2006). This suggests that the dopaminergic system is involved in tic-related OCD. However, our data suggest that the *COMT 158Met* allele is not a common etiologic factor for tic-related OCD and TD.

The *COMT Met158Met* genotype is associated with a lower score on the vocal subscale of the YGTSS. This suggests that the *COMT Met158Met* genotype may be protective for vocal tics.

Previous studies did not find a difference in YGTSS score between patients with different genotypes (Lim et al., 2009; Tarnok et al., 2007). However, these studies did not investigate YGTSS subscales.

In conclusion, our study suggests that specific etiological factors might be involved in specific tic symptom dimensions. *BNDF 66Met* allele is associated with GTS in females. Further studies are needed to confirm and extend our current findings.

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# Screening of the epsilon sarcoglycan gene in Tourette syndrome and obsessive compulsive disorder

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Mutations in the epsilon sarcoglycan gene (*SGCE* gene) are associated with obsessive compulsive behavior in some families with myoclonus-dystonia (Saunders-Pullman et al., 2002). Therefore, it seemed of interest to screen patients with familial obsessive compulsive disorder (OCD) and genetically related tic disorders such as Tourette syndrome (GTS) and chronic motor tic disorder (CMT) for mutations in the *SGCE* gene.

Two studies previously investigated the *SGCE* gene in GTS patients. One of these studies screened 32 patients with GTS and comorbid OCD (de Carvalho Aguiar et al., 2004), the other study screened 83 sporadic GTS patients (Asmus et al., 2005b). Both studies did not find pathogenic mutations.

The current study investigates the *SGCE* gene in 96 patients (39 women, 57 men, mean age 36±12; range 9–65) with OCD and/or GTS, or CMT. OCD was diagnosed according to diagnostic and statistical manual of mental disorders fourth edition (DSM-IV) criteria using the Structured Clinical Interview for DSM-IV axis 1 disorders. GTS and CMT were diagnosed according to DSM-IV criteria. Tic symptoms and tic severity were assessed using the Yale Global Tic Severity Scale.

Ninety-two patients with a positive family history for tics and/or obsessive compulsive symptoms were screened. Twenty eight of these patients had OCD, 26 patients had OCD and comorbid GTS, 35 patients had GTS, two patients met the criteria for CMT but not GTS or OCD, and one patient had OCD and CMT. Four patients had sporadic OCD. DNA was obtained from venous blood or buccal swabs. Eleven exons and flanking intronic sequences of the *SGCE* gene were amplified by PCR and PCR products were sequenced on an automatic sequencer.

No mutations were detected in the coding region. Five variants were found in intronic regions. One of these is new (c.1253+54T>C) and was found in a GTS patient from Iranian origin. As no Iranian controls were available, we screened 112 chromosomes from the Dutch population and the variant was not present. The variant was also absent in patient's son with GTS. The other in-tronic variants (c.232+134G>A, c.390+39_40dupGT, c.391–43A>C and c.391–3T>C) are known polymorphisms.

Three new 3' untranslated region sequence variants were found. We screened 220 control chromosomes from the Dutch population for these variants. Two (c.1314+85T>C and c.1314+101A>G) were also found in control chromosomes. The third variant (c.1314+172T>C) was found in a female patient with OCD and GTS and her son with

probable GTS. It was, however, not found in her daughter with OCD nor in 220 control chromosomes and its frequency was not significantly different between cases and controls (Fischer's exact test; p=0.466).

In conclusion, we did not find any evidence for apparently deleterious variants of the coding and flanking intronic region of the *SGCE* gene in a sample of familial cases with OCD and/or GTS or CMT. The functional relevance of a newly found c.1314+172T>C 3' untranslated region variant has yet to be determined.

Acknowledgement: This work was supported in part by the Tourette Syndrome Association

Chapter 8

# Summary and discussion

**Summary**

Obsessive-compulsive disorder (OCD) is characterized by intrusive, unwanted thoughts (obsessions) and/or repetitive behaviors (compulsions), and has a lifetime prevalence of about 2-3% (Bebbington, 1998; Karno et al., 1988). Tourette's disorder (GTS) is a tic disorder characterized by the presence of multiple motor tics and at least one vocal tic. The prevalence of GTS has been estimated between 1:100 to 1:10.000 (Keen-Kim and Freimer, 2006).

A genetic predisposition for OCD and GTS has been widely acknowledged. However, the search for susceptibility genes for both disorders has been hampered by negative and inconsistent results (Hemmings and Stein, 2006; Keen-Kim and Freimer, 2006; Pauls, 2003).

An important factor contributing to the limited success in the identification of genetic susceptibility genes for OCD and GTS may be their symptomatic and genetic heterogeneity.

In the research described in thesis, an attempt is made to reduce the symptomatic heterogeneity of OCD and GTS by performing analyses using the various symptom dimensions of these disorders.

Chapter 2 describes an item-level factor analysis on the YBOCS-symptom checklist (YBOCS-CL) data of the largest sample of OCD patients to date, which yielded 5 Obsessive-Compulsive (OC) symptom dimensions:

Factor 1: Taboo – Sexual, aggressive and religious obsessions; Factor 2: Contamination obsessions and cleaning compulsions; Factor 3: Doubts – (obsessions related to fears of having caused harm to self or others, doubting and checking compulsions related to these fears); Factor 4: Rituals/superstition, and Factor 5: Symmetry/hoarding. An important difference with other item-level factor analyses is that somatic items loaded on different factors and leaving out somatic items provided a better fit. Heritability (39%) accounted for a large part of the variance. Indeed, all these factors except for the rituals and superstition factor were shown to be highly heritable. All factors were negatively associated with age of onset, whereas the taboo factor and the taboo factor was associated with sex. The taboo factor and the symmetry/hoarding factor were associated with age of assessment. However, sex, age of onset and age of assessment accounted only for a small amount of variance of the factor scores.

The latent class analysis described in chapter 3 yielded three or five classes. The classes represent increasing frequency of endorsement of the different symptoms; except for one class in the five-class model with high endorsement of contamination obsessions and cleaning compulsions. Classes with higher endorsement were predominantly male, had a lower age of onset and a higher rate of comorbid tic.

In chapter 4, symptom dimensions obtained by factor analysis on the YBOCS-CL are used as quantitative phenotypes in an association study of the *BDNF Val66Met* polymorphism. The role of the *BDNF Val66Met* polymorphism in OCD and its phenotypic expression is studied. Females with the *Met66Met* genotype had a higher age of onset and a trend for lower symptom severity. Patients with the *Val66Val* genotype had a higher score on a symptom dimension with sexual and religious obsessions. These results suggest that the *BDNF Met* allele has a protective role in females and that the *BDNF Val66Val* genotype may modulate the symptom dimension with sexual and religious obsessions.

In chapter 5 the role of the *COMT Val158Met* polymorphism in OCD and its phenotypic expression including YBOCS-CL symptom dimensions is studied.

There was a trend for an association of the *COMT 158Met* allele with OCD in males, and an interaction between the *COMT Val158Met* genotype and sex on the somatic and sensory phenomena symptom dimension, with females showing lower scores.

In our association analysis of the *COMT Val158Met* and the *BNDF Val66Met* polymorphisms and GTS/CMT described in chapter 6, a case-control approach was used. An association was found between the *BDNF 66Met* allele and GTS/CMT in females. However, this association was not shown using a family-based (TDT) approach. This could be due to the limited power of the TDT, or, alternatively, the positive result could be caused by population stratification. Item-level factor analysis of Yale Global Tic Severity Scale (YGTSS) symptom checklist scales yielded 6 factors: Factor 1: Simple motor tics, aggressive behavior, words and palilalia; Factor 2: Complex motor tics and tic-related compulsions: Factor 3: Throat clearing and eye tics; Factor 4: Whistling, mouth and nose tics, animals and bird noises and syllables; Factor 5: Facial, head and neck tics with coughing, sniffing and syllables and Factor 6: Complex shoulder movements and echolalia.

No association was found between mean score per item for any of the factors and genotypes or alleles of the *BDNF Val66Met* or the *COMT Val158Met* polymorphism. However, there was a trend for a lower score on the vocal subscale of the Yale Tic Severity scale in patients with the *COMT Met158Met* genotype compared to patients with other genotypes suggesting a protective role for the *COMT Met158Met* genotype in the severity of vocal tics.

Chapter 7 describes a mutation screening of the *SGCE* gene in which no apparent deleterious mutations in the *SGCE* were identified. A variant in de 3' UTR region has been found in a female patient with GTS and OCD and her son with probable GTS, but not in her daughter with OCD.

**Discussion**

Both OCD and GTS are relatively common disorders, likely to be caused by multiple genes, each with a limited effect on disease risk. Molecular genetic studies in OCD and GTS to date have yielded inconsistent results. This may be caused by the genetic and phenotypic heterogeneity of OCD and GTS or by the limited effect sizes of the genes involved.

We tried to reduce phenotypic heterogeneity by identifying symptom dimensions by item-level factor analyses on symptom checklists for obsessive-compulsive symptoms and tics. We also used other phenotypes such as age of onset and symptom severity, family history and comorbidity of OCD and GTS/Tics.

Since the effect sizes of the genes involved in OCD and GTS are likely to be small, large samples sizes are needed to detect these genes. Large part of the molecular genetic studies in OCD and GTS to date probably had too limited power due to limited sample sizes. The research described in this thesis involves large multicenter studies with relatively large sample sizes. Although we had relatively large sample sizes, the power to detect recessive effects of rare variants and sex-specific effects was still limited. However, using dimensional phenotypes increases power to detect associations (Silverman and Palmer, 2000).

Genome-wide approaches such as genome wide association (GWA) studies do not require prior knowledge about the disease under investigation. Nor are they limited to coding DNA and also pick up non-coding variants such as copy number variations (Plomin and Davis, 2009). A disadvantage of GWA studies is the large sample sizes needed, up to cohorts of thousands of patients, to obtain enough power to detect susceptibility regions with genome-wide significance estimates that are generally regarded as evidence for a susceptibility region (Psychiatric GWAS Consortium Coordinating Committee, 2009). Therefore, because of the sample sizes of the cohorts of patients in our study, we chose a candidate gene approach; using case-control association studies to detect effects of common variants in some candidate genes. In addition, we used mutation screening in another candidate gene to detect probable rare variants.

Another factor probably contributing to the inconsistent results of genetic association studies in OCD and/or GTS is population stratification. Family-based association studies have been developed to prevent population stratification (Schulze and McMahon, 2002). Family-based association studies require recruitment of parent-child trios. Parents were not available for most OCD patients and a lot of GTS patients in our cohorts. Moreover, the power of family based association studies is lower than the power for case-control studies. Therefore, we chose a case-control design.

Genomic control or stuctured association methods have been developed to adjust for possible population stratification (Pritchard and Donnelly, 2001). These methods use genotypes of unlinked marker loci and therefore require more extensive genotyping. Therefore, we chose to limit the possibility for population stratification by collecting cases and controls by choosing controls from the same geographical regions.

The symptomatic heterogeneity of OCD and Tic disorders

In order to reduce phenotypic heterogeneity we performed item-level factor analysis on the Yale-Browne Obsessive-Compulsive Scale symptom checklist (YBOCS-CL) and the Yale Global Tic Severity Scale symptom checklist data of a large group of patients in order to identify symptoms dimensions of obsessive-compulsive symptoms and tics respectively.

The item-level factor analysis on YBOCS-CL data described in chapter 2 of this thesis supports a five factor model for the symptomatology for OCD. The fact that all of the factors except for the rituals/superstition factor were shown to be heritable suggests that four of these factors may constitute heritable phenotypes which may be used in future genetic studies. The rituals/superstition factor encompasses the miscellaneous items of the YBOCS symptom checklist. These data suggest that this factor (along with its items) might as well be omitted from the YBOCS symptom checklist for genetic studies, since it does not appear to be heritable, and, thus, will not be of value in genetic studies. Thus, our results suggest that the genetic factors underlying OCD constitute one single factor, as well as several genetic factors that are etiologically distinct. Since some of these factors have been shown to form etiologically distinct entities, they may be included as distinct symptom dimensions in genetic studies. The DSM-V is likely to include these symptom dimensions as subtypes of OCD (Leckman et al., 2007).

The fact that a better fit was observed by omitting the somatic items from the analysis suggests that somatic items are heterogeneous. Some of the somatic items may be associated with OCD, whereas other may be etiologically unrelated to OCD. Therefore, future research has to be performed to investigate if, and if so which somatic symptoms are to be considered part of the OCD phenotype.

The results of the latent class analysis described in chapter 3 further support the validity of one underlying genetic susceptibility factor for OC symptoms.

A limitation of the studies investigating the structure of tic symptoms, obsessions and compulsions is that studies to date have not included the different OC symptoms/symptom dimensions and tic symptoms/symptom dimensions. Future research performing factor analysis using both the YBOCS-CL and the YGTSS-CL in a large cohort of patients with OCD and/or tic disorders should directly target the unraveling of the association between the different tics, obsessions and compulsions.

To further distinguish different types of obsessions one may distinguish ego-dystonic "exogenous" obsessions from ego-syntonic "endogenous" obsessions. Moreover, one may refine symptom dimensions by including goal directedness of the repetitive behavior to distinguish between compulsions, performed to reduce anxiety and non-anxiety-related repetitive "impulsions", since repetitive behaviors in Tourette patients are less frequently anxiety related than repetitive behaviors in OCD (Cath et al., 2001).
The factor structure found on the Yale Global Tic Severity Scale symptom checklist described in chapter 6 is difficult to interpret. The Yale Global Tic Severity scale has been validated. However, its accompanying symptom checklist has not. Future efforts should be directed to the development and validation of an extensive checklist of motor and vocal tics.

Brain-derived neurotrophic factor gene (*BDNF*)
The functional *BDNF Val66Met* polymorphism was chosen for a candidate gene studies in OCD and GTS, based on its effect on activity dependent BDNF secretion and its previously described associations between this polymorphism and OCD.
Although the present studies support a role for *BDNF Val66Met* variant in the phenotypic expression of both OCD and GTS, the mechanisms involved may be different.
There is accumulating evidence that life events play a role in the etiology of OCD (Gothelf et al., 2004; Maina et al., 1999). Deviations in basal activity and reactivity of the hypothalamic-pituitary-adrenal (HPA) axis have been described in OCD as well as in GTS (Corbett et al., 2008; Kluge et al., 2007). Sex differences have been described for the PHA axis responsivity (Kudielka and Kirschbaum, 2005). Moreover, the *BDNF Val66Met* polymorphism has been shown to exert a sexual dimorphic effect on cortisol response to moderate social stress (Shalev et al., 2009). Men with the *BDNF Val66Val* genotype show a higher response to moderate social stress compared to males with the *BDNF Val66Met* genotype, whereas females with the *BDNF Val66Met* genotype showed a higher response compared to females with the *BDNF Val66Val* genotype (Shalev et al., 2009). The mechanism with which the *BDNF Val66Met* polymorphism exerts a sexual dimorphic effect on OCD symptoms may therefore be HPA axis mediation in response to stress caused by life events. Studies investigating the interaction of the *BDNF Val66Met* polymorphism and life events on the risk for OCD or OC symptom dimensions are warranted to investigate this.
Childhood sexual abuse has been found to constitute a risk factor for development of OCD (Caspi et al., 2008). It is interesting to speculate that the nature of the adverse life event may be related to the nature of the obsessive-compulsive symptoms. For example that

sexual abuse may cause sexual obsessions. This could explain the association of the *BDNF Val66Met* polymorphism with a symptom dimensions that included sexual obsessions.
In contrast to OCD, life events do not seem to play a major role in the etiology of GTS (Horesh et al., 2008). Therefore, mechanism for the role of *BDNF Val66Met* polymorphisms in the etiology of GTS other than stress-medicated PHA-axis responsivity may be involved. GTS patients show age related age and sex-dependent differences in brain-morphology compared to normal controls (Peterson et al., 2001b). The *BDNF Val66Met* polymorphism affects age-related changes in brain morphology in a sex-dependent manner (Nemoto et al., 2006). Therefore, the role *BDNF Val66Met* polymorphism in the etiology of GTS in females may be mediated by age related changes in brain morphology.

Catechol-*O*-methyl transferase gene (*COMT*)
The dopaminergic system has been implicated in both GTS and OCD (Denys et al., 2004b; Rampello et al., 2006). The functional *COMT Val158Met* polymorphism was chosen for candidate gene studies in OCD and GTS based on its influence on the degradation of dopamine.
The trend for an association between the *COMT 158Met* allele and OCD in males in our study is in concordance with a recent meta-analysis (Pooley et al., 2007). A recent study showed reduced COMT expression in peripheral lymphocytes of patients with OCD, especially in males (Wang et al., 2009). This supports a gender-specific effect of COMT in OCD.
In accordance with previous studies, we found no evidence for an association between the *COMT* gene and tic disorders. However, the influence of the *COMT Val158Met* variant on vocal tic severity suggests a modifying influence of this polymorphism on vocal tics.

Epsilon sarcoglycan gene (*SGCE*)
Both symptomatic and asymptomatic carriers of mutations in the *SGCE* gene in some families with Myoclonus-Dystonia (M-D) show an increased frequency of OCD (Hess et al., 2007; Saunders-Pullman et al., 2002). Therefore, the *SGCE* gene has been considered a candidate gene for OCD. We performed a mutation screening for the *SGCE* gene in a cohort of patients with OCD and/or GTS. Taken together, our and previous studies (Asmus et al., 2005b; de Carvalho Aguiar et al., 2004) suggest that mutations in the *SGCE* gene do not account for a large proportion of the cases of GTS and OCD. However, a limitation of the studies was that PCR based screening was performed, which does detect large deletions including complete exons. It has been increasingly acknowledged that large deletions account for a considerable number of mutations in the *SGCE* gene (Asmus et al., 2005a;

Grunewald et al., 2008; Han et al., 2008). Therefore, future studies should include gene doses studies in order to be able to pick up large deletions within the *SGCE* gene such as Multiplex Ligation Probe Amplification (MLPA).
The variant in the 3' UTR of the *SGCE* gene in one of the patients lies in a putative micro RNA binding region. Therefore, this variant could have a micro RNA mediated influence on *SGCE* expression and further study of the influence of this variant in *SGCE* gene expression and its role in GTS and OCD are warranted.

**Future prospects in the search for other susceptibility genes for OCD and GTS**
Candidate gene studies in OCD/GTS based on current hypotheses of the pathophysiology of OCD and GTS have had only limited success. Further candidate gene studies based on these hypotheses seem only to be useful if phenotypical heterogeneity and methodological limitations are appropriately addressed.
Researchers should realize that (some of) the current hypotheses of the pathophysiology of OCD may be only applicable only to certain subtypes of OCD. In molecular genetic studies to date, only limited use has been made of OCD subtypes. Only hoarding and early-onset OCD have been widely used as phenotypes. Our research suggests that the following symptom dimensions taboo, contamination/cleaning, doubts and symmetry/hoarding are heritable phenotypes that may be used in future genetic studies in OCD. However, since a one-factor model had a reasonable fit, total symptom count and YBOCS severity score seem to be heritable, a general genetic susceptibility also seems to exist. Therefore, OCD as a nosological entity still seems to be a valid phenotype. In future genetic studies of OCD both OCD as a nosological entity as well as symptom dimensions should be used as phenotypes. The phenotypical expression of GTS and tic disorders (TD) in general has to be more intensively investigated in order to identify meaningful subtypes. The hoarding and early-onset OCD phenotype have already successfully used in linkage and association studies. Successful uses of subtypes in other disorders are linkage studies of language, social responsiveness and repetitive behavior in autism (Losh et al., 2008).
Since multiple genes are involved in the pathways that are hypothesized to be involved in OCD and/or GTS, genetic studies in OCD and GTS should move towards studies investigating multiple genes, for example multiple genes in neurotransmitter pathways or immunological processes suspected to be involved in (subtypes) of OCD and/or GTS. Moreover, gene-gene interaction studies are warranted. Specifically, gene-gene interaction studies between genes in the same pathway (e.g. the dopaminergic, serotonergic or glutamatergic) are warranted. To date, only two gene-gene interaction studies have been published for OCD, both with negative result focusing on the interaction between *BDNF* and the gene for its specific receptor, the *NTRK2* gene (Alonso et al., 2008a), and the

interaction between the *BDNF Val66Met* polymorphism and polymorphism in the *5HTTP* gene (Wendland et al., 2007). The negative results of these studies may have been caused by their relatively limited sample sizes. Alternatively, the negative results may be caused by the fact that both studies did not take into account gene-environment interactions. Two studies investigating gene-gene interaction between polymorphism in the *BDNF* and *5HTTP* gene in depression only found an interaction between these genes in the risk for depression when life events were considered in the analyses (Kaufman et al., 2006; Kim et al., 2007).

Since the current hypotheses of the pathophysiology of OCD and TD do no seem to explain the whole spectrum of these phenotypes, genome-wide approaches are warranted to identify new susceptibility regions and genes. Molecular genetics studies of OCD and GTS are likely to shift towards genome wide association studies (GWAS) with large sample sizes of several thousands of patients. GWAS studies in other complex diseases such as diabetes mellitus, cardiovascular diseases and cancer with appropriate sample sizes have been successful (Cichon et al., 2009). In these studies, most findings involved novel susceptibility genes or regions. GWAS studies thus seem to be able to provide new clues in the search for pathophysological mechanisms in disease. GWAS studies for both OCD and GTS are currently underway. Genomic control or structured association methods should be used to correct for possible population stratification. If susceptibility genes are discovered, functional gene networks can be used to select candidate genes for future genetics studies in OCD and GTS. Functional gene networks are networks that connects genes known to share a common function and can be used to predict new candidate genes from known susceptibility genes (Franke et al., 2006; Lehner and Lee, 2008).

An alternative approach to identify new candidate gene for OCD and GTS is the study of gene-expression. In micro array gene expression studies the expression of many genes are studied at the same time. Differences in gene expression between patients with GTS and/or OCD and healthy individuals may provide us with clues in the identification of pathways involved in the pathophysiology of OCD and/or GTS. This is difficult for GTS and OCD. Since the biological substrate for OCD and GTS involves the Central Nervous System, it is difficult to obtain appropriate tissue sample to investigate gene expression. Tissue available for human gene-expression studies will be post-mortem tissues. This causes limitations due to the possible influence of several disease unrelated factors such as post mortem delay and cause of death on gene-expression (Ferrer et al., 2008). Second, when differences in gene expression are found between patients with GTS and/or OCD and controls, these differences can be either cause or consequence of the disorder and additional research is warranted to investigate the cause and consequence of these differences in gene-expression.

A micro-array gene-expression in post-mortem putamen tissue of GTS patients has been performed (Hong et al., 2004). Among the genes differentially expressed in GTS tissue were genes protein tyrosine phosphatase (PTP) family, proteins known to play a role in de dopaminergic and serotonergic systems (Hong et al., 2004). In a microarray study investigating gene expression in blood there was no evidence for a unique gene expression profile in GTS. However, subgroup analysis showed that a group of genes involved in immunological processes were differentially expressed in GTS patients. Cluster analysis showed a higher number of GTS patients in the higher expression group compared to the control subjects consistent with the PANDAS hypothesis that auto-immune processes may be involved in the etiology of GTS in a subgroup patients (Tang et al., 2005). To our knowledge, no micro-array gene expression studies have been performed in OCD.

Despite the fact that OCD and TD seem to be heritable phenotypes, alternative phenotypes may be more successfully used in genetic studies of OCD and/or TD, Objective and quantifiable biological markers representing neurobiological processes which may cause or characterize OCD and/or GTS may be more closely related to the genetic susceptibility genes for these disorders than the traditional clinical diagnosis. Such biological markers form intermediate phenotypes called endophenotypes (Gottesman and Gould, 2003). In order to constitute an endophenotype a biological marker must be heritable, associated with disorder in the population; its presence should be independent of disease-state and has to co-segregate with the disorder within families (Gottesman and Gould, 2003). Examples of possible endophenotypes for OCD and GTS are neuro-anatomic anomalies, neurochemical anomalies and neurocognitive functioning. For example, white matter anomalies in the right inferior parietal region and the right medial frontal region have been shown in patients with OCD and unaffected first-degree relatives (Menzies et al., 2007). Executive function tests showed longer reaction times and lower scores in executive function tests as well as cognitive inflexibility and motor impulsivity in relatives of patients with OCD compared to healthy controls (Chamberlain et al., 2007; Delorme et al., 2007). Increased gray matter in the parieto-singulo-striatal system was positively correlated with motor inhibitory control whereas increased gray matter density in the frontal system was negatively correlated with motor inhibitory control. Gray matter densities in the parieto-singulo-striatal region and in the frontal system in patients were correlated with those in their family members (Menzies et al., 2007). These findings suggest that neuro-imaging and neurocognitive testing or a combination may provide endophenotypes that could be used in genetics studies. However, monozygous twins discordant for OC symptoms showed differences in brain activation correlated with executive function between the twin scoring high and the twin scoring low on obsessive compulsive symptoms (den Braber et al., 2008). This suggests that these differences in brain activation are mediated by environmental influences and illustrates that

investigation the heritability of putative endophenotypes is warranted. To our knowledge, only one association study using endophenotypes has been described in OCD. Association between glutamatergic concentration in the anterior cingulate and occipital cortex and polymorphisms in the GRIN2B and glutamate transporter gene was investigated (Arnold et al., 2009). Glutamate concentration in the anterior cingulate cortex was associated with a functional promoter variant in the GRIN2B glutamate receptor gene in pediatric OCD (Arnold et al., 2009).

Gene-gene interaction studies, gene-environment interaction studies and a combination of these studies may also provide us with more insight in the pathophysiology of GTS and/or OCD. These approaches have been successful for other disorders. For example, studies in ADHD showed an interaction between a polymorphism in exon 5 of dopamine transporter gene and a history of smoking during pregnancy for severe combined type ADHD (Todd and Neuman, 2007).
Ultimately, genotype, environmental factors and markers of biological pathways can be modeled. For example, BDNF, early life stress and arousal pathway measures have been modeled in a regression model. No main effects of BDNF genotype were found, but there was an interaction of BDNF genotypes and early life stress gave reduced hippocampal gray matter and lateral prefrontal cortex volume and higher depression (Gatt et al., 2009).
We are currently investigating the influence of the interaction between *BDNF Val66Met* variant and early life trauma on OCD.

In conclusion, despite the tremendous efforts, molecular genetic studies in OCD and/or GTS have had only limited success in identifying susceptibility genes and regions. Future studies should be directed at defining relevant subtypes of OCD and/or GTS or relevant endophenotypes. Large cohorts of patients should be recruited in order to be able to perform suitably powered studies.
This approach may increase the chance of identification of new susceptibility genes for (subgroups of) GTS and/or OCD. This may provide us with insights in new pathophysiological mechanisms of (subgroups of) GTS and OCD. These insights in the pathophysiology may provide tools for the refinement of diagnosis and treatment of OCD and GTS.

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# Nederlandse samenvatting

Obsessieve-compulsieve stoornissen (OCS) worden gekenmerkt door ongewenste gedachten en/of beelden (obsessies) en/of repetitief gedrag (compulsies). Ongeveer 2-3% van de mensen voldoet tijdens zijn of haar leven aan de criteria voor de diagnose OCS. Het syndroom van Gilles de la Tourette (GTS) is een ticstoornis die gekenmerkt wordt door de aanwezigheid van ongewenste bewegingen (motorische tics) en vocale uitingen (vocale tics). De diagnose GTS wordt gesteld als er sprake is van meerdere motorische tics en ten minste één vocale tic. Het syndroom van Gilles de la Tourette komt bij ongeveer 0,5% van de wereldbevolking voor.
Uit familie- en tweelingstudies blijkt dat bij sommige vormen van OCS en GTS erfelijke aanleg een rol speelt. Verder blijkt dat sommige vormen van OCS en GTS genetisch aan elkaar verwant zijn. De zoektocht naar genen die betrokken zijn bij deze aandoeningen is echter tot nog toe weinig succesvol gebleken. Een belangrijke factor die de zoektocht naar genen die bij OCS en/of GTS betrokken zijn mogelijk bemoeilijkt, is de verscheidenheid aan symptomen (fenotypische heterogeniteit) bij deze aandoeningen. Verder zijn bij de erfelijke aanleg van OCS en GTS meerdere genen betrokken. Individuele genen hebben slechts een relatief klein effect, waardoor grote groepen patiënten moeten worden bestudeerd om deze effecten aan te kunnen tonen.
In het eerste hoofdstuk van dit proefschrift worden de genetische studies die zijn uitgevoerd bij OCS en GTS beschreven. Hierbij wordt een overzicht gegeven van de studies van kandidaatgenen. Kandidaatgenen zijn genen die op grond van hun functie of hun positie in het genoom mogelijk een rol spelen bij het krijgen van de ziekte of bij het verloop van de ziekte. In de studies van kandidaatgenen wordt gekeken of verschillende varianten (allelen) van deze genen samenhangen met de ziekte. Ook kan worden gekeken naar de rol van verschillende genotypen. Een genotype is de combinatie van de twee allelen die van beide ouders zijn overgeërfd. Erft men van beide ouders hetzelfde allel dan wordt men homozygoot voor dit allel genoemd, erft men van beide ouders een verschillende variant van hetzelfde allel dan wordt men heterozygoot genoemd.
Uit het overzicht van de kandidaatgenstudies bij OCS en GTS bleek dat de meest veelbelovende kandidaatgenen voor genetische studies afkomstig zijn uit het dopaminerge, serotonerge en het glutamaterge systeem.
Dat is niet verwonderlijk omdat bij (sommige vormen van) OCS en GTS een verhoogde activiteit van het dopaminerge systeem in de hersenen een rol speelt. Bij (sommige vormen van) OCS speelt daarnaast een verstoring in het serotonerge en het glutamaterge systeem waarschijnlijk ook een rol.

In het eerste deel van dit proefschrift (hoofdstuk 2 en 3) worden studies beschreven waarbij gekeken werd naar de fenotypische heterogeniteit van OCS.
De gouden standaard om symptomen van OCS in kaart te brengen is de Yale-Brown Obsessive-Compulsive Severity scale symptom checklist (YBOCS-CL). Dit is een lijst

waarmee de aanwezigheid of afwezigheid van 74 verschillende symptomen (obsessies en compulsies, ingedeeld in 13 categorieën) van OCS wordt gescoord.
Hoofdstuk 2 beschrijft een factoranalyse van YBOCS-CL gegevens van een grote groep OCS patiënten. Deze studie is verricht met behulp van data uit vijf verschillende instituten wereldwijd, in het kader van een internationaal genetisch consortium. Met deze factoranalyse werd gekeken welke symptomen vaak voorkomen en een symptoomdimensie vormen.
In tegenstelling tot de meeste eerdere factoranalyses van de YBOCS-CL werd naar afzonderlijke symptomen (op symptoomniveau) gekeken in plaats van naar de symptoomcategorieën (categorie-niveau).
De factoranalyse leverde 5 Obsessieve-Compulsieve (OC) symptoomdimensies op:
Factor 1: Taboe – Seksuele, agressieve en religieuze obsessies;
Factor 2: Smetvrees en wasdwang;
Factor 3: Twijfel – Obsessies gerelateerd aan angst om zichzelf of anderen schade te berokkenen of te hebben berokkend, twijfel en controledwanghandelingen die hier mee samenhangen;
Factor 4: Rituelen/achterdocht;
Factor 5: Symmetrie/verzamelen.
Een belangrijk verschil met andere factoranalyses op symptoom-niveau is dat somatische obsessies en daarmee samenhangende compulsies op verschillende factoren laadden en het weglaten van deze symptomen tot duidelijker te interpreteren resultaten leidde. Erfelijkheid verklaarde een groot deel (39%) van de variatie in de YBOCS-CL data. Scores op alle symptoomdimensies behalve de symptoomdimensie rituelen/bijgeloof waren voor een belangrijke mate erfelijk. Erfelijkheid verklaarde 28% van de variatie in de score op de symptoomdimensie taboe, 35% van de score op de symptoomdimensie smetvrees/wasdwang, 40% op de symptoomdimensie twijfel en 37% van de scores op de symptoomdimensie symmetrie/verzamelen. De ernst van de symptomen werd voor 52% door erfelijke factoren verklaard.
In hoofdstuk 3 werd latente klasse analyse uitgevoerd op YBOCS-CL data. Hierbij werden OCS patiënten aan de hand van de aanwezigheid van de verschillende symptomen in verschillende groepen ingedeeld. De analyse leverde een indeling in drie of in vijf groepen patiënten op. Deze groepen patiënten verschilden in de frequentie waarin alle symptomen voorkwamen. De groepen werden niet gekenmerkt door hoge scores op specifieke symptomen. Uitzondering was één groep patiënten in het 5-klassen model, die vooral hoog scoorden op smetvrees en wasdwang. Patiënten uit de groepen die werden gekenmerkt door een hogere frequentie van symptomen, hadden een lagere leeftijd bij het begin van de symptomen en hadden vaker tics. In deze groep zaten bovendien meer mannen dan in de andere groepen.

In het tweede deel van dit proefschrift wordt de rol van kandidaatgenen bij OCS en GTS bestudeerd.
De rol van twee genetische varianten (polymorfismen) die aangrijpen op deze systemen bij OCS, GTS en hun symptoom dimensies werd eveneens bestudeerd.
In hoofdstuk 4 werd de rol van de *BDNF Val66Met* variant in het gen dat codeert voor Brain-Derived Neurotrophic Factor (het *BDNF* gen) in OCS en de uitingsvormen ervan, zoals scores op de symptoomdimensies die werden gevonden bij factor analyse, bestudeerd. BDNF stimuleert de ontwikkeling van zenuwcellen, onder andere van zenuwcellen in het serotonerge systeem. De *Val66Met* variant vermindert de hoeveelheid BDNF die vrijkomt.
Vrouwen met twee *66Met* varianten (het *Met66Met* genotype) hadden een hogere leeftijd bij begin van de symptomen en minder vaak familieleden met obsessieve-compulsieve symptomen. Vrouwen met het *Val66Val* genotype hadden ernstiger symptomen. Patiënten met het *Val66Val* genotype hadden een hogere score op een symptoom dimensie met seksuele en religieuze obsessies. Deze resultaten suggereren dat het *BDNF Met* allel een beschermende werking heeft bij vrouwen en dat het *BDNF Val66Val* genotype een rol speelt bij de symptoomdimensie met seksuele en religieuze obsessies.
In hoofdstuk 5 wordt de rol van de *Val158Met* variant in het catechol-*O*-methyl transferase (het *COMT* gen) bij OCS en zijn uitingsvormen zoals YBOCS-CL symptoomdimensies bestudeerd. Catechol-*O*-methyl transferase (COMT) is een enzym dat betrokken is bij de afbraak van onder andere dopamine. De *Val158Met* variant zorgt voor een verminderde activiteit van dit enzym.
Er was een trend voor een associatie van het *COMT 158Met* allel met OCS bij mannen en er was een interactie tussen het *COMT Val158Met* genotype en sekse op de scores op de symptoomdimensie met somatische en sensorische fenomenen, waarbij vrouwen lagere scores hadden.
Hoofdstuk 6 beschrijft een onderzoek van het *COMT Val158Met* en het *BDNF Val66Met* polymorfisme en ticstoornissen. Er werd een associatie gevonden tussen het *BDNF 66Met* allel en ticstoornissen bij vrouwen.
Verder werd factor analyse gedaan op de tic symptomen van de Yale Global Tic Severity Scale symptom checklist (YGTSS-CL). De YGTSS-CL is een vragenlijst waarmee de aanwezigheid of afwezigheid van verschillende tics worden gescoord.
De factoranalyse leverde 6 factoren op:
Factor 1: Enkelvoudige motorische tics, agressief gedrag, het uitspreken van woorden en palilalie (het herhalen van eigen woorden en zinnen of delen daarvan);
Factor 2: Complexe motorische tics en tic-gerelateerde compulsies;
Factor 3: Keelschrapen en oogtics;
Factor 4: Fluiten, mond- en neustics, het maken van dier- en vogelgeluiden en het uitspreken van lettergrepen;

Factor 5: Aangezichts, hoofd- en nektics, kuchen, snuiven en het uitspreken van lettergrepen;
Factor 6: Complexe schouderbewegingen en echolalie (anderen naspreken).
Er was geen verband tussen de gemiddelde score per symptoom voor de verschillende symptoomdimensies en genotypes of allelen van het *BDNF Val66Met* of het *COMT Val158Met* polymorfisme. Patiënten met het *COMT Met158Met* genotype neigden echter naar een lagere score op de schaal voor vocale tics in vergelijking met patiënten met andere genotypes. Dit suggereert dat het *COMT Met158Met* genotype beschermd tegen vocale tics.
Hoofdstuk 7 beschrijft een mutatie screening van het *SGCE* gen. Mutaties in dit gen zijn beschreven in families met myoclone dystonie (M-D). Dit is een ziektebeeld met kortdurende schokkende bewegingen (myclonieën) en psychiatrische stoornissen, waaronder OCS. In families met M-D en een mutatie in het *SGCE* gen was de mutatie in het *SGCE* gen geassocieerd met OCS.
Het *SGCE* gen werd bestudeerd in een groep patiënten met voornamelijk een familiare vorm van GTS en/of OCS. Er werden in deze groep geen duidelijke ziekte-veroorzakende mutaties gevonden. Wel werd een variant gevonden in een deel van het gen dat niet codeert voor het SGCE eiwit, maar mogelijk wel betrokken is bij de regulatie van de mate waarin het gen tot expressie komt. Deze variant werd gevonden in een patiënte met GTS en OCS. Bij een zoon van deze patiënte met tics werd de variant ook gevonden. De variant werd echter niet gevonden in de dochter van deze patiënte waarbij OCS is gediagnosticeerd. Verder onderzoek naar het belang van deze variant lijkt aangewezen.

Aangezien erfelijkheid zowel een rol speelt op het aantal aanwezige symtpomen op de YBOCS-CL en de ernst van de symptomen en de scores op vier van de vijf symptoomdimensies, lijkt er sprake te zijn van zowel een erfelijke factor voor OCS in het algemeen als erfelijke factoren voor specifieke symptoomdimensies.
Voor toekomstige genetische studies van OCS lijkt het gebruik van een combinatie van de diagnose OCS en symptoomdimensies een goede benadering te zijn. De erfelijkheid van de gevonden symptoomdimensies zal echter nog verder moeten worden onderzocht om te bepalen welke symptoomdimensies erfelijk zijn en welke symptoomdimensies het beste in genetische studies kunnen worden gebruikt.
Het identificeren van symptoomdimensies bij GTS staat nog in de kinderschoenen. Verder onderzoek zal duidelijk moeten maken of en zo ja welke symptoomdimensies er zijn bij GTS.
Een andere benadering is het gebruik van endofenotypen. Endofenotypen zijn biologische of neuropsychologische kenmerken die door erfelijke factoren worden beïnvloedt, samen met de ziekte voorkomen en een uiting zouden kunnen zijn van de processen de schakel vormen tussen genen en de ziekte.
Verder zouden bij de genetische studies bij OCS en GTS naast kandidaatgenstudies ook genoom-wijde associatiestudies moeten worden verricht. Hiervoor zijn echter grote groepen patiënten nodig. Uit deze genoomwijde associatiestudies komen mogelijk nieuwe kandidaatgenen naar voren. Bij toekomstige studies zal ook rekening gehouden moeten worden met interacties tussen verschillende genen en tussen genen en omgevingsfactoren.
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